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Investigations on Plasmin

IV. Some Experiments on Activation

GUNNAR LUNDBLAD

Wenner-Gren's Institute for Experimental Biology, University of Stockholm, Stockholm, Sweden

The activation mechanism of plasmin, the proteolytic enzyme in blood, is still rather obscure in spite of several investigations. On the basis of recent work carried out by Christensen and MacLeod [1], Loomis *et al.*[2], Permin [3], and Macfarlane and Pilling [4], the following nomenclature now seems to be generally accepted.

Plasminogen is the inactive precursor present in the euglobin fraction.

Antiplasmin is the plasmin inhibitor, present in the albumin fraction and removable with chloroform.

Antiplasminogen — the naturally occurring plasminogen inhibitor that prevents the conversion of plasminogen to plasmin.

Streptokinase is the exotoxin produced by certain strains of hemolytic streptococci, catalysing the conversion of plasminogen to plasmin.

Fibrinokinase — activating factor(s) in certain tissue cells.

Among the investigations on activating and inhibiting factors the following works may be mentioned. Streptokinase and fibrinokinase are considered to promote the conversion of plasminogen to plasmin directly, while chloroform destroys the plasmin inhibitor after which plasminogen autocatalytically is transformed slowly to plasmin (Christensen [5]). Ungar and Mist [6] observed an increase in fibrinolytic activity in serum from normal guinea pigs by mixing either with peptone, agar, hyaluronic acid, chondroitin sulfuric acid, glycogen or small amounts of heparin. This activation of plasmin required the presence of some serum factor which is non-precipitable with the euglobin fraction and is destroyed by heating at 56° C. Guillaumie [7] found a reduction of the proteolytic inhibiting power in serum after heating to 56° C, and Grob [8] found that the heating of diluted serum at 80° C resulted in a better medium for bacterial

growth. It is believed that this is at least partly due to the destruction of the serum antiprotease. Herberts [9] has reported an increase in proteolytic activity in lung extracts from shocked guinea pigs on heating to 60° C. In all these cases the activation seems to occur through the destruction of the plasmin inhibitor. Grob [10] found that both reducing and oxidizing agents decreased the proteolytic activity. Hultin and Lundblad [11] probably proved the existence of antiplasminogen, which is assumed by Loomis *et al.*[2].

This paper will report some experiments on activation by oxidizing agents together with some results obtained from experiments with plasmin inhibitor.

EXPERIMENTAL

The plasmin was prepared from bovine serum, according to Loomis *et al.*[2], treated with chloroform and lyophilized. The proteolytic activity was measured viscosimetrically on gelatin at 35.5° and calculated according to Hultin's formula as reported earlier by the present writer [12]. The dry plasmin preparation was stored at — 20° C and dissolved in physiological saline immediately before use. A one per cent solution of plasmin in saline was used. The N-content of this solution, which was called P500, was 0.075 per cent. The plasmin inhibitor was prepared according to Permin [3] and from the same serum as the plasmin. The original activity of the plasmin solution P500 was 4.25×10^{-9} per ml and after treatment with streptokinase 6.15×10^{-9} per ml, which is about 37 per cent activation. As oxidizing agents were used 3.0 per cent hydrogenperoxide and 0.002 *N* iodine solution. In the activation experiments the solution P500 was not treated with streptokinase. 1.00 ml P500 and 0.50 ml of either oxidizing solution or water were incubated 90 minutes at 20° C and then mixed with 3.0 ml of 4.0 per cent gelatin of pH 7.20. Table 1 shows the results from two different series.

Table 1. Activation by oxidation.

Expt. no.	Added	pH in mixture	Activity
1	H_2O	7.10	1.85
2	H_2O_2	7.05	4.13
3	I_2-KI	7.10	4.41
4	H_2O	7.12	1.61
5	H_2O_2	7.07	2.81
6	I_2-KI	7.08	3.11

Thus the plasmin is activated ca. 1.7—2.2 times by hydrogen peroxide and 1.9—2.4 times by iodine. Now, in order to see if this activation is due

to the destruction of the remaining plasmin inhibitor, this was prepared according to Permin [3] as shown in table 2, and its influence upon P500 was investigated. This is shown in table 3.

Table 2. Preparation of plasmin inhibitor.

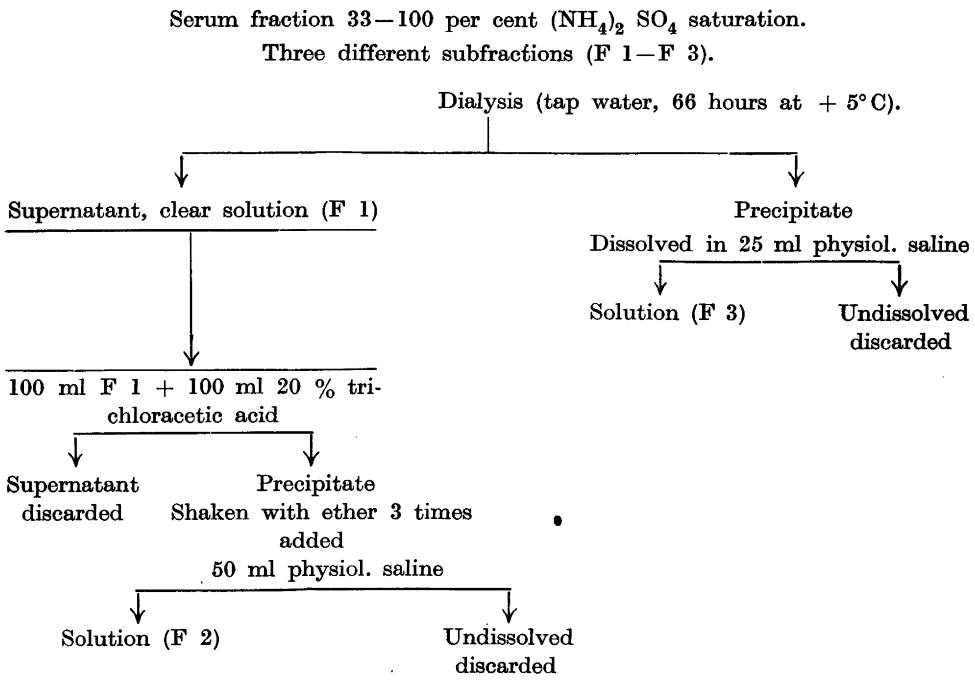

Table 3. Influence of the fractions on plasmin.

Each sample which consisted of 1.00 ml P500 and 0.50 ml of added substance was incubated 90 min. at 20° C and then mixed with 3.00 ml 4.0 per cent gelatin.

Expt. no.	Added	pH in mixture	Activity
7	H_2O	7.12	1.98
8	F 1	7.10	0.00
9	F 2	7.01	1.51
10	F 3	7.11	5.34

These results show the complete inhibition by the untreated plasmin inhibitor in F 1, the power of which is strongly deminished when precipitated with trichloracetic acid. It is astonishing to see the great plasmin content in

F 3, which is the remaining globin fraction evidently not precipitated at the plasmin preparation, and which perhaps explains the low plasmin activity of several other preparations.

If the activating power of the oxidants used in Table 1 is due to the destruction of plasmin inhibitor present, the treatment of F 1 with the oxidants should take away the inhibiting power of the plasmin inhibitor. The experiments shown in Table 4 were therefore performed.

Table 4. Treatment of plasmin inhibitor.

1.00 ml P500 and added solution were mixed after incubation with 3.00 ml of 4.0 per cent gelatin. In exp. 14 and 15 F 1 was treated for 1 hour at 20° C, before addition to P500 and incubation at 20° C.

Expt. no.	Added solution	Incubation time	pH in mixt.	Activity
11	0.50 ml H_2O	90 min.	7.04	4.8
12	0.50 ml H_2O + 0.50 ml H_2O_2	90 »	6.90	26.7
13	0.50 ml F 1	90 »	7.02	0.0
14	0.50 ml F 1 + 0.50 ml H_2O_2	30 »	6.82	1.0
15	0.50 » F 1 + 0.50 » I_2-KI	30 »	7.04	0.2

We notice the strong activation by hydrogenperoxide in this series and we see that the treatment of F 1 abolishes its inhibiting power only to a low extent, which shows that the activation of plasmin by oxidizing agents is not caused by the destruction of the plasmin inhibitor.

Whether the activation partly depends on the influence upon the formation of plasmin from plasminogen, and partly upon destruction of the anti-plasminogen or yet another factor, cannot be judged until further investigations have been carried out.

SUMMARY

The proteolytic activity of a plasmin solution is increased by oxidizing agents such as hydrogenperoxide and iodine.

The inactivating power of the plasmin inhibitor does not considerably diminish on the treatment of the plasmin inhibitor with these agents.

Thus the activation of the plasmin by these oxidizing agents is not obtained by the destruction of the plasmin inhibitor, but by another factor.

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REFERENCES

1. Christensen, L. R., and MacLeod, C. M. *J. Gen. Physiol.* **28** (1945) 559.
2. Loomis, E. C., George, C. Jr., and Ryder, A. *Arch. Biochem.* **12** (1947) 1.
3. Permin, P. M. Dissertation. Copenhagen 1949.
4. Macfarlane, R. G., and Pilling, J. *Lancet* **251** (1946) 562.
5. Christensen, L. R. *J. Gen. Physiol.* **30** (1946) 149.
6. Ungar, G., and Mist, S. H. *J. Exp. Med.* **90** (1949) 39.
7. Guillaumie, M. *Compt. rend. soc. biol.* **136** (1942) 783.
8. Grob, D. *J. Gen. Physiol.* **26** (1943) 431.
9. Herberts, G. *Acta Soc. Med. Usal.* **54** (1949).
10. Grob, D. *J. Gen. Physiol.* **33** (1949) 103.
11. Hultin, E., and Lundblad, G. *Acta Chem. Scand.* **3** (1949) 620.
12. Lundblad, G. *Acta Chem. Scand.* **3** (1949) 354.

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