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Investigations on Plasmin

I. On the Proteolytic Activity of Plasmin

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As the nomenclature of the fibrinolytic enzyme in blood plasma has been very unclear, a short survey of the nomenclature may be appropriate. The enzyme has been called plasma proteas, plasma trypsin (Schmitz[1]), lysin factor (Milstone[2]), tryptas (Ferguson[3]), fibrinolas (Dyckerhoff[4]), plasma proteolytic enzyme (Ratnoff[5]), fibrinolysin (Kaplan *et al.*[6]; Loomis *et al.*[7]), and finally plasmin suggested by Christensen and MacLeod[8]. The name plasmin is to prefer to fibrinolysin as the latter has been used also for the activator from hemolytic Streptococci, the streptokinase (Christensen[9]). The precursor of plasmin is consequently called plasminogen[8] and the inhibitor present in the albumin fraction of plasma is called antiplasmin by Macfarlane and Pilling[10].

The proteolytic activity of plasmin has been determined in different ways. Christensen[11] chose as the unit of activity that amount plasmin in a volume of 0.2 ml that will lyse a standard fibrin clot in 30 minutes at pH 7.2—7.3 and a temperature of 40° C. Later Christensen[9] determined the activity viscosimetrically on gelatin according to the formula: $\frac{(V_0 - V_{10}) \cdot 100}{V_0 \cdot 10}$, where V_0 is the initial specific viscosity and V_{10} is the viscosity at 10 minutes, the temperature being 37.0° C and at pH 7.4. Kaplan *et al.*[6] measured the decrease in viscosity of a 4 per cent gelatin solution after one hour at 37.5° C at different pH values in phosphate- and imidazol buffers. Loomis *et al.*[7] defined one unit of plasmin activity as »that amount which will dissolve one ml of 0.3 per cent fibrin clot in 120 seconds at pH 7.2 and 45° C in an isotonic saline system buffered with imidazol».

Recently Hultin[12] has introduced an expression for enzymic activity, first experimentally tested on the degradation of starch by α-amylase[13]. Later the

proteolytic activity of pepsin, gelatinase, and trypsin upon gelatin [14] was determined by means of this formula.

The expression is:

$$A^{t^\circ}_{e/s} = C_s^2 \cdot \frac{\mathrm{d}\frac{1}{\eta_{sp}}}{\mathrm{d}t}$$

where

A enzymic activity in units per gram of solution at t° C
$^e/_s$ abbreviation for enzyme and substrat
c_s concentration of substrate in grams per gram of solution
η_{sp} specific viscosity and
t time in seconds

As the results seem to confirm the formula and as the reproducibility is good, it seems appropriate to use the formula for measuring the proteolytic activity of plasmin. In some viscosity measurements it is necessary to use an extended formula taking the ionic factor in account. That has recently been done by Ingelman and Malmgren [15] in calculating enzymic breakdown of polymetaphosphate. In this investigation however no ionic factor has been introduced as all the experiments have been performed under the same conditions.

The plasmin was prepared from ox blood, the fibrin was removed by stirring and subsequent straining, the erythrocytes were removed by centrifugation, and the serum was treated according to Loomis *et al.*[7]. The plasmin, which is in the euglobulin fraction, was precipitated between 25 and 29 per

Table 1. Activity of different plasma fractions.

No.	Batch	Fraction	Per cent N	Activity: $A \cdot 10^6$/g N	Storage
1	I	25—29	3.54	63.2	—
2	I	25—29	3.54	49.9	60 days, 18°, dry
3	II	0—25	10.3	41.1	1 ½ hours, 18°, sol.
4	II	0—25	10.3	32.8	7 » 18°, »
5	II	0—25	10.3	21.7	11 » 18°, »
6	II	0—25	10.3	23.9	90 days, 18°, dry
7	II	25—29	8.76	62.6	—
8	III	15—25	3.81	29.8	—
9	III	25—29	8.34	58.2	—
10	III	29—33	9.11	20.9	—
11	III	15—25	3.81	21.7	1 year, 2°, dry
12	III	25—29	8.34	16.1	1 » 2°, »
13	III	29—33	9.11	5.8	1 » 2°, »

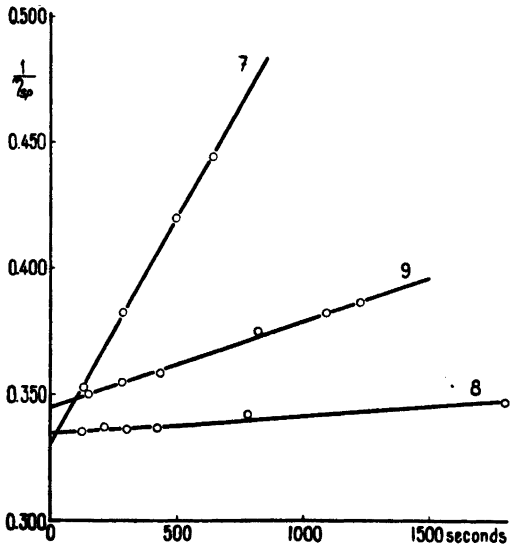

Fig. 1. The enzymic activity calculated from the slope of the line.

cent ammonium sulfate saturation. The antiplasmin was abolished by shaking with chloroform for half an hour. For comparison the fractions obtained before 25 and after 29 percent saturation with ammonium sulfate were also tested. The samples were made up of 2.00 ml plasmin solution (5.0 per cent dry weight) and 5.00 ml of a 3.00 per cent gelatin solution at 35.5° C and pH 7.2. The outflow times were measured in Ostwald viscosimeters.

As can be seen in Table 1 there is also enzymic activity in the fractions before 25 and after 29 per cent saturation with ammonium sulfate. The experiments no 2 and 6 show that there is a certain loss of activity in plasmin even when kept in dry state.

The experiments nos. 7, 8 and 9 from Table 1 are shown in Fig. 1. As can be seen the inverse values of the specific viscosity are proportional to the time in accordance with the formula.

After storing one year the loss of activity is less in the profraction (15—25 per cent). The reason for this might be due to a different content of plasminogen. Dyckerhoff [4] found that after a plasmin solution had stood for 48 hours at room temperature, no activity remained in the solution. Experiments nos. 3, 4 and 5 show a decrease in activity of 4—5 per cent per hour.

In order to find out something about the stability of a plasmin solution the following experiments were carried out.

A one per cent (dry weight) solution of plasmin (batch III, fraction 25—29, one year old) was used, the nitrogen content in the solution being 0.042 per

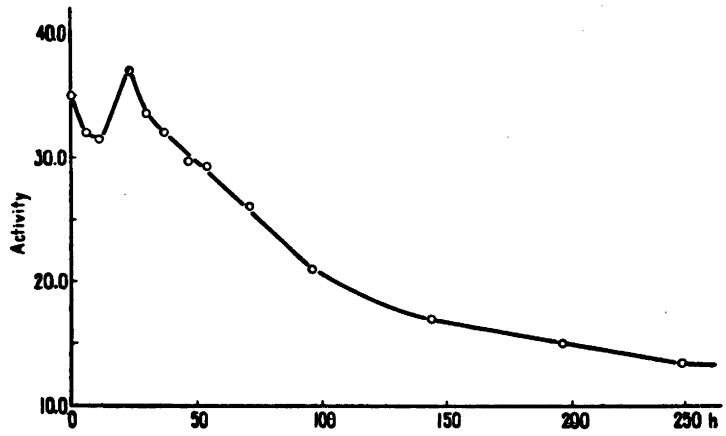

Fig. 2. Influence of time upon the proteolytic activity of a plasmin solution.

cent. From this solution stored at 18° C one ml samples were taken at different times. Each assay sample consisted of 1.00 ml plasmin and 3.00 ml of a 4.00 per cent gelatin solution and each sample was run at 35.5° C for about 75 hours. The pH was 7.3 chosen in the range of maximum stability — pH 7.2—7.4 — which for plasmin is the same as the range of maximum activity according to Christensen and MacLeod [8]. Controls with inactivated plasmin (30 min boiling) were constant.

Table 2. Activity of a plasmin solution.

Age of solution in hours	Comparative activity
0	5.21
8	3.96
24	3.86
50	5.28
78	4.03
170	3.47

Table 2 shows that after 170 hours there is still about 50 per cent of the original activity in the solution. A very strange fact is that after 50 hours the activity is increasing again and then decreasing. This gave reason for a repeated investigation of the influence of time upon a plasmin solution. Therefore a new series was started, each sample with a control of inactivated plasmin, and the

result can be seen in Fig. 2. The increase in activity after a certain time occurs also in this series. The explanation of this phenomenon may be due to the presence of the proenzyme plasminogen in the beginning, the plasminogen being activated in some way. Therefore besides the decreasing activity of the plasmin present from the beginning there is also during a certain time an increased activity due to new formed plasmin. When all plasminogen is converted into plasmin there is only the decreasing activity. The resulting activity when measured thus gives a course like that in Fig. 2.

A discussion of this phenomenon in connection with the mathematical treatment of the problem will appear in another paper in this journal.

SUMMARY

The activity and stability of some plasmin preparations are calculated according to a recently introduced expression.

A strange phenomenon in the influence of time upon the proteolytic activity of a plasmin solution is reported.

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