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Investigations on Plasmin

III. On the Formation of Plasmin from Plasminogen

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In a previous paper in this journal [1] one of us reported the strange behaviour of plasmin solutions in several experiments. At first, the proteolytic activity decreased normally, but after a certain time the solutions revealed an activity greater than that of the fresh solution. The activity thereupon again decreased normally.

THEORETICAL

The increase in the activity of a plasmin solution gives raise to two different hypotheses. In both cases we must presume the presence of plasminogen in the plasmin solution, but the mechanism of the plasmin formation will be quite different if we postulate the absence or the presence of an antiplasminogen, which must first be destroyed before the plasminogen can be transformed into plasmin. We will first consider the case when we have plasmin and plasminogen but no antiplasminogen.

The mechanism of the decomposition of the plasminogen may first be assumed to be autocathalytic as in the trypsin formation from trypsinogen. This case has been treated by Kunitz and Northrop [2, 3]. The activity first increases more and more rapidly and after a while decreases slowly. There is no activity minimum so the behaviour of the plasmin solutions cannot be explained in this way.

A reaction may also be postulated to proceed in such a way that the enzyme and the enzymogen are both assumed to decompose according to a reaction of the first order. We apply the following notations:

A = the enzyme activity,
E_0 = the enzymogen amount at zero time (one unit of enzymogen gives one unit of enzyme),
k = the rate constant for the inactivation of the enzyme,
c = the rate constant for the transformation of the enzymogen to enzyme, and
t = the time.

For the enzyme activity we get the following differential equation

$$\frac{dA}{dt} = cE_0e^{-ct} - kA \tag{1}$$

which has the following solution if $c \neq k$

$$A = \frac{cCe^{-kt} - cE_0e^{-ct}}{c - k} \tag{2}$$

where C is an integration constant. If the activity at zero time is A_0, we get

$$A = A_0e^{-kt} + E_0 \frac{e^{-kt} - e^{-ct}}{1 - \frac{k}{c}} \tag{3}$$

If the time is counted so that the activity at zero time is zero, we get the following expression:

$$A = E_0 \frac{e^{-kt} - e^{-ct}}{1 - \frac{k}{c}} \tag{4}$$

If $c = k$, we get

$$A = E_0kte^{-kt} \tag{5}$$

and if $c = \infty$, we get

$$A = E_0e^{-kt} \tag{6}$$

The curves of the equations (4—6) are given in Fig. 1. It can easily be shown that the curves have a maximum at the point where they are cut by the line $A = E_0e^{-kt}$, *i. e.* $k/c = 0$, and no other maximum or minimum points.

From this treatment we gather that the mechanism assumed in this calculation is not valid for an enzyme showing one minimum and one maximum in its activity.

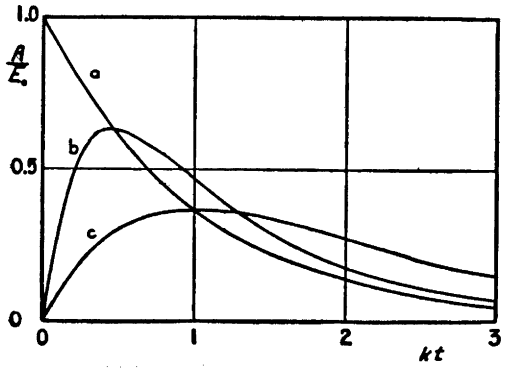

Fig. 1. The activity of an enzyme solution, where one unit of enzymogen is transformed to enzyme in a reaction of the 1st order with the rate constant c, and the enzyme is inactivated in a reaction of the 1st order with the rate constant k.
a. $c = \infty$. *b.* $k/c = \frac{1}{4}$. *c.* $c = k$.

If, however, we postulate the presence of an antiplasminogen, we may assume that this antiplasminogen can be broken down by the plasmin. If the antiplasminogen is present in excess compared with the plasminogen, a great part of the antiplasminogen must first be broken down before the plasminogen can be transformed into plasmin. During this first step, the plasmin inactivation is likely to proceed normally. When the last residues of the antiplasminogen are distroyed, the plasminogen is rapidly transformed into plasmin. When this plasmin formation is completed, the inactivation of the plasmin again proceeds normally. In the activity determinations, however, the antiplasminogen is diluted with the substrate, and so the probability of a given linkage in the antiplasminogen being split is greatly decreased. Thus the activity at the moment when the plasmin solution was mixed with the substrate solution is measured.

EXPERIMENTAL

The experimental values from measurements reported in a previous article by one of us [1] have been recalculated in the way recently described by us [4]. We have thus found the activity values listed in Table 1. The activity values are given for the first and the second step of the break down of gelatin [5].

The initial viscosity of the gelatin solution changed during the experiment, and so we must assume that the later determinations in this series cannot without hesitation be compared with the earlier ones. This is also reflected by the fact that the proportion between the activity values obtained from the first and from the second step of break down of the gelatin solution is not constant. The interesting part of the activity determinations were, however, all performed before the gelatin solution had changed very much. Hence

Table 1. The activity of a plasmin solution at different times, indicating the presence of an antiplasminogen.

Time hours	Gelatin $\frac{1}{\eta_{sp}}$	Activity (μA)	
		1st step	2nd step
0	1.08	0.022_5	0.0074
6	1.11	0.021	0.0070
11	1.06	0.019	0.0066
23	1.15	0.024_5	0.009
30	1.20	0.023	0.009
37	1.33	0.022	0.009
47	1.29	0.020	0.0077
54	1.26	0.018_5	0.0082
71	1.46	0.015	0.0078
96	1.48	0.012_5	0.0065
144	1.67	0.009_5	0.0067
196	1.57	0.009	0.0051
244	1.61	0.007	0.0041

The function $1/\eta_{sp}$ of the initial viscosity of the gelatin solution in each activity assay is given in the second column.

this series may be used to support the hypothesis of the occurence of an antiplasminogen *.

SUMMARY

1. A mathematical treatment is given for the activity of an enzyme solution, where an enzymogen is transformed to an enzyme in a reaction of the first order and the enzyme is also inactivated in a reaction of the first order.

2. Several hypothetical mechanisms for the transformation of plasminogen to plasmin are discussed. Earlier observations showing that the activity of plasmin solutions after a preliminary period of decrease, suddenly increases and thereupon again decreases cannot be explained either as an autocathalytic reaction or as a spontanous decomposition of the plasminogen in a reaction of the first order.

3. The behaviour of the plasmin solutions may, however, be explained by the presence of an excess of antiplasminogen that prevents the transformation of plasminogen to plasmin, and which must first be broken down by the plasmin, before the transformation can take place.

* Called by Loomis *et al.*[6] antiprofibrinolysin.

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