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Investigations on Plasmin

II. On the Determination of the Activity of Instable Enzyme Solutions

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In an investigation recently published by one of us[1] certain difficulties arose in the determination of the activity of an enzyme, plasmin, originating from the fact that the time necessary for the assay was so long and the stability of the enzyme so low that the activity considerably declined during the assay.

In order to render the calculations of enzymic activity more accurate under such conditions, we have established the following mathematical treatment for this problem.

THEORETICAL

In an enzymatic hydrolysis, the number of linkages broken per unit time is directly proportional to the amount of enzyme if the amount of substrate present is sufficiently large and if the affinity of the enzyme to the substrate is considerable. This means that if the enzyme is stable, the number of linkages broken per unit time is always the same. If the activity of the enzyme declines, we can still use the ordinary formulas, if the time is changed into such a function of the time that the number of linkages broken is proportional to this function.

This function of the time will depend on the law of decomposition of the enzyme. This law may be complicated. In the beginning, however, the decomposition may usually be considered as a reaction of the first order. Under these assumptions the problem will be treated with the following notations:

A_t = the enxyme activity at the time t,
c = the proportional factor for the enzymic break down,
C = an integration constant,

k = the rate constant for the inactivation of the enzyme,
n = the number of linkages,
n_0 = the number of linkages originally present,
t = the time, and
$T_{\frac{1}{2}}$ = the half-life period of the enzyme.

The activity of the enzyme under these conditions is

$$A_t = A_0 e^{-kt} \tag{1}$$

Further

$$dn = -cA_0 e^{-kt} \cdot dt \tag{2}$$

$$n = -cA_0 \frac{e^{-kt}}{-k} + C \tag{3}$$

If $t = 0$ we have $n = n_0$. Hence $C = n_0 - \frac{cA_0}{k}$ and

$$n = n_0 - cA_0 \frac{1 - e^{-kt}}{k} \tag{4}$$

If this expression is compared with the corresponding expression for stable enzymes,

$$n = n_0 - cA_0 t \tag{5}$$

we gather that the time should be changed into the function

$$\frac{1 - e^{-kt}}{k}$$

The problem is now to find the rate constant for the inactivation of the enzyme. The present authors have employed the method of successive approximations.

From equation (1) we get

$$\log A_t = \log A_0 - kt \log e \tag{6}$$

and

$$k = -\frac{\Delta \log A_t}{\Delta t \cdot \log e} \tag{7}$$

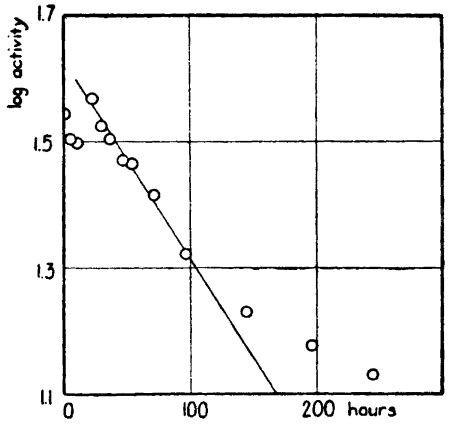

Fig. 1. Graph for the calculation of the rate constant of the inactivation of the enzyme.

and the half-life period

$$T_{\frac{1}{2}} = -\frac{\log 2 \cdot \Delta t}{\Delta \log A_t} \tag{8}$$

EXPERIMENTAL

We have applied the expressions evaluated in the theoretical part to the measurements of plasmin activity reported in the article mentioned above [1]. From the data given there on plasmin activity (*cf.* Fig. 2) we have prepared the present Fig. 1, giving the logarithm of the enzyme activity as a function of the time.

From the graph in Fig. 1 we have calculated $k = 0.0075$ and $T_{\frac{1}{2}} = 93$ (time in hours).

The three last points of the activity determinations lie considerably above the straight line. This may indicate that the stability increases with continued inactivation. If a straight line is fitted to these points and the corresponding calculations of k and $T_{\frac{1}{2}}$ are made, the following values are obtained: $k = 0.0023$ and $T_{\frac{1}{2}} = 300$ (time in hours).

The rate constant for the enzyme inactivation determined in this way cannot be assumed to be accurately valid for the inactivation in the reaction mixture with gelatin. For want of better values at the present early stage of plasmin investigation, this value may however be assumed to be at least of the right magnitude. Hence we have calculated the function $(1-e^{-kt})/k$ with this value. An example of the result is given in Fig. 2.

From the figure we gather that the time may advantageously be corrected according to the given function. It is possible to fit 2 straight lines to the

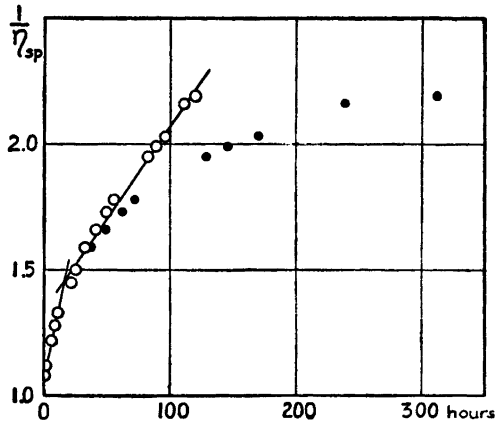

Fig. 2. Viscosimetric assay of plasmin with gelatin. Correction of the time for the spontaneous inactivation of the enzyme. Dots give the uncorrected time. Circels give the corrected time $= (1-e^{-kt})/k$.

measurements as described by one of us [2,3] and thus calculate the enzyme activity for two different steps of the hydrolysis.

For the assay, 1 ml of plasmin solution was mixed with 3 ml of 4 % gelatin solution. Hence [4], remembering that the time was counted in hours, and taking 0.0235 for the derivative of the first line (Fig. 2), the activity in μA units is

$$\tfrac{4}{1} \cdot \tfrac{3}{4} \cdot \tfrac{3}{4} \cdot 0.04 \cdot 0.04 \cdot 0.0235 \cdot \frac{1}{3600} \cdot 10^6 = 0.0235$$

SUMMARY

The spontanous inactivation of an enzyme during the assay can be corrected for by substituting the following function of the time t and the rate constant k for the enzymic inactivation: $(1-e^{-kt})/k$ for the reaction time. The application of the formula is exemplified by the viscosimetric assay of plasmin.

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