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General kinetics of enzyme cascades

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The theory of the kinetics of enzyme cascades is developed. Two types of cascades are recognized, one in which the products are stable (*open cascades*) and another in which the products are broken down (*damped cascades*). It is shown that it is a characteristic of a cascade that the final product appears after a certain lag phase. After this lag phase, the velocity of product formation can be very rapid. It is shown that whereas open cascades will always show a complicated time–product relation, damped cascades can under certain circumstances resemble a simple enzymic reaction. Because the relation between the over-all reaction velocity in the extrinsic coagulation cascade and the concentration of any of the proenzymes in this cascade is a hyperbolic one, it is concluded that this cascade is of the damped type rather than the open type.

The kinetics of a single step enzymic reaction

An enzyme cascade can be defined as a sequential array of enzymic reactions in which the product of one reaction serves as the enzyme in the next. A discussion of the kinetics of an enzyme cascade can best start with the consideration of a single enzymic reaction. This subject, of course, can only be treated superficially here. For a more detailed discussion the reader is referred to textbooks (Dixon & Webb 1965; Gutfreund 1965).

We can write an enzymic reaction as follows:

$$S \xrightarrow{E} P \tag{A}$$

and the most simple kinetic formula that can be thought of is found when there is so much substrate that the reaction velocity is proportional to the enzyme concentration. In that case

$$v = kE \tag{1}$$

(v = reaction velocity; k = a constant; E = enzyme concentration).

This relationship is encountered only under very special circumstances.

A more generally applicable picture of the situation is given by the Michaelis–Menten model, which depicts the state of affairs in an enzymic reaction as

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftarrows}} C \xrightarrow{k_{+2}} E + P. \tag{B}$$

Mathematical evaluation of this model is best done with approximations. The best

known approximation is the so called Briggs–Haldane formula which gives v as

$$v = \frac{k_{+2} ES}{K_m + S} \tag{2}$$

(S = substrate concentration) where

$$K_m = \frac{(k_{-1} + k_{+2})}{k_{+1}}. \tag{3}$$

This formula is valid only under the following conditions

(*a*) $S \gg E$. Computer simulation experiments (Hemker & Hemker 1967, unpublished results) have shown that the error introduced by this approximation will remain below 1 % as long as $S > 30E$.

(*b*) *The 'transient phase' can be neglected.* The transient phase is defined as the lapse of time immediately after the start of the reaction during which there is a net synthesis of the enzyme substrate complex.

(*c*) *Initial reaction velocities are measured.* This means that during the period over which the reaction velocity is measured, S does not drop considerably.

With respect to the question of whether we can maintain these assumptions in the steps of the reaction systems we are interested in here, it must be remarked that:

(*a*) The assumption $S \gg E$ is not a very realistic one, because very often we do not even know whether a certain reactant should be regarded as a substrate or as an enzyme. On the other hand, we can be reasonably sure that either $S \gg E$ or $E \gg S$, because one of the two reactants is the reaction product of the foregoing step. We were able to show that under these conditions (Hemker, Hemker & Loeliger 1965*a*)

$$v = \frac{k_{+2} ES}{K_m + E + S} \tag{4}$$

which for the case in which $E \gg S$, reduces to

$$v = \frac{k_{+2} ES}{K_m + E}. \tag{5}$$

This is equal to the Briggs–Haldane formula except that S in the denominator is replaced by E. This place in the formula is apparently reserved for the concentration of the reactant present in excess. If we define the substrate in an unorthodox way as the reactant that is present in excess, we can maintain the Briggs–Haldane formula. But we should keep in mind that if we do so, we do not differentiate between the reaction schemes:

$$S_1 \overset{E_1}{\rightarrow} E_2 \qquad S_2 \overset{E_2}{\leftarrow} E_3 \tag{C}$$

and

$$S_1 \overset{E_1}{\rightarrow} P_1 \qquad P_1 \overset{E_2}{\rightarrow} E_3 \tag{D}$$

(*b*) To neglect the transient state, which is the second assumption in the Briggs–Haldane case, does not seem to be very realistic either. Although it is

justified in the majority of cases where the reaction is started by adding a fixed amount of enzyme at zero time, in our systems we are dealing with enzyme concentrations that change in time, and there is no good reason to assume that the transient phase can be neglected under these circumstances.

Gutfreund (1965) gave a solution for the kinetics of the Michaelis–Menten model providing for recognition of the transient phase. With a small modification, this yields the analogue of the Briggs–Haldane formula valid for the transient phase

$$v = \frac{k_{+2}ES}{K_m+S}(1-e^{-bt}), \tag{6}$$

where

$$b = k_{+1}(K_m+S). \tag{7}$$

Here we can recognize the Briggs–Haldane formula multiplied by a factor which is 0 at $t = 0$ and which will tend to become 1 when t grows large.

(*c*) The third assumption of the Briggs–Haldane treatment, i.e. the consideration of initial reaction velocities, will have to be maintained because we can not yet handle the mathematics if we discard it. Moreover, we know that experimental results do not force us to drop this assumption.

To summarize, we have three formulae for the reaction velocity as a function of reaction constants and concentration of reactants in a one step enzymic reaction, namely:

$$(a) \quad v = kE, \tag{1}$$

$$(b) \quad v = \frac{k_{+2}ES}{K_m+S}, \tag{2}$$

$$(c) \quad v = \frac{k_{+2}ES}{K_m+S}(1-e^{-bt}). \tag{6}$$

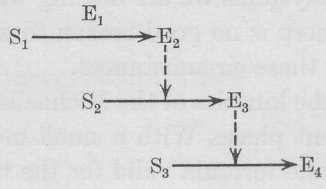
It is evident that the more realistic the formula, the more complicated it becomes.

The kinetics of cascade systems

The only thing intuition tells me when I try to visualize what happens in an enzyme cascade is that it will necessarily be quite a bit more complicated than the kinetics of a single step reaction. This compels a thorough mathematic investigation of the subject. The outcome of such a study will very probably be a set of more or less complicated formulations (i.e. mathematical equations) that are only of limited comfort to those—the author included—who try to investigate the enzyme cascade system at the laboratory bench, unless such equations are translated into terms that can be checked at the bench. This is what we have tried to do here.

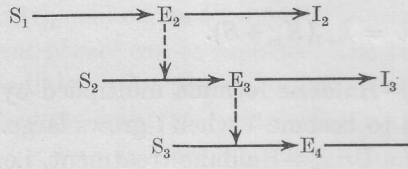
In the first place, it should be recognized that there are actually two different classes of cascade systems, which we will call the open and the damped cascades.

(*a*) *The open cascade* is the cascade as originally proposed by Macfarlane.



(E)

(*b*) *The damped cascade* is a cascade in which each enzyme is broken down as soon as it appears



(F)

From the standpoint of physiology, the damped type represents an interesting case because many active principles generated in cascades are known to be inactivated.

Kinetics of the open cascade

The best thing to do seems to be to apply the most realistic formulae for the reaction velocity of one step to each of the steps in the cascades. As you will see later, this results in the most horrible and unhelpful formulae. We will therefore discuss the results of applying the most simple of the single step formulae first. When an open cascade is triggered by the addition of a fixed amount of enzyme at zero time, the reaction velocity of the first step will be $v_1 = k_1 E_1$. The concentration of the product of the first step will therefore rise linearly in time according to the formula:

$$P_{1t} = tv_1 = tk_1 E_1 \tag{8}$$

and, because the product of the first step is the enzyme of the second step, it follows that

$$E_{2t} = tk_1 E_1. \tag{9}$$

The velocity of the second step will therefore be

$$\mathrm{d}P_2/\mathrm{d}t = v_{2t} = k_2 E_{2t} = tk_1 k_2 E_1. \tag{10}$$

Integration shows that the concentration at time t of the product of the second step will be

$$P_{2t} = \tfrac{1}{2} t^2 k_1 k_2 E_1. \tag{11}$$

For a cascade of n steps this becomes

$$P_{nt} = (1/n!)\, t^n E_1 \prod_{i=1}^{n} k_i. \tag{12}$$

So the course of product formation in an open cascade will be a higher degree curve of the type given in figure 1. Some important conclusions can be drawn from this

simple formula. In the first place, the most important effect of a cascade is not an amplification but a triggering effect. Just as a sneeze can trigger an avalanche, the first addition of enzyme triggers a burst of product formation after a certain incubation time (see also Hemker 1965).

The actual amount of product present at a given time is determined by all the multiplied reaction constants and E_1, that is by the term

$$(1/n!)E_1 \prod_{i=1}^{n} k_i.$$

There is no reason to assume that this will be a big number. When, for instance, all these constants are smaller than one, we end with a very small number (figure 2).

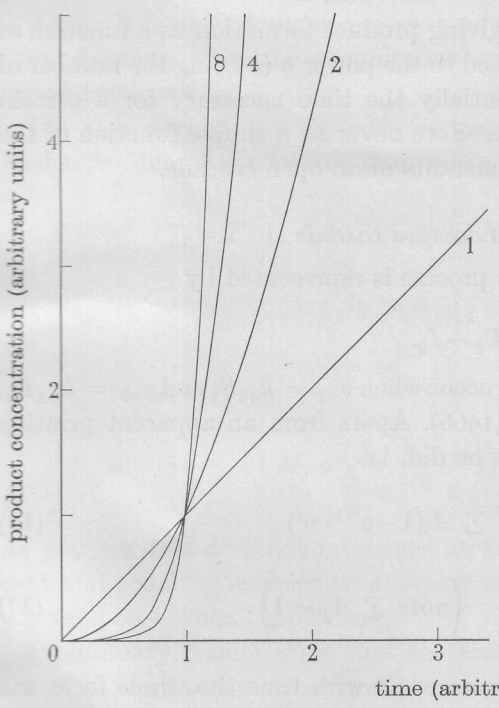


FIGURE 1

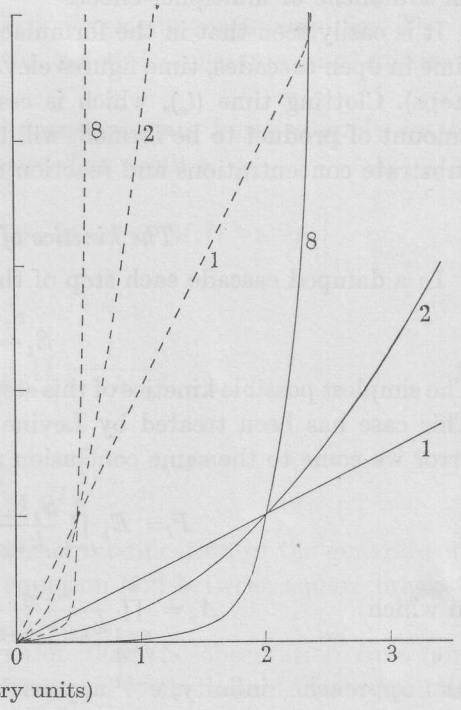


FIGURE 2

FIGURE 1. Product formation curves in an open cascade when all reaction constants are unity. The figures indicate the step number (n).

FIGURE 2. Product formation curves in an open cascade when the reaction constants differ from unity. The figures indicate the step number (n). Solid lines: $k = 0{\cdot}5$; dashed lines $k = 2{\cdot}0$.

The conclusion must therefore be that an open enzyme cascade is an avalanche rather than an amplifier or, with the model of a photomultiplier tube in mind, we should perhaps call it a multiplier system rather than an amplifier.

When we calculated the formulae that would result if we started with a more sophisticated formula for the one step reaction velocity, we found that, using the

Michaelis–Menten model and not neglecting the transient phase, we obtained

$$P_t = E_1 \prod_{j=1}^{n} \frac{S_j k_{+2j}}{S_j + K_{mj}} \left[\sum_{m=1}^{n} \left\{ \Sigma \left(-\frac{1}{b_j} \right)^{n-m} A_j \right\} \frac{t^m}{m!} + \sum_{j=1}^{n} \left(-\frac{1}{b_j} \right)^n A_j \left(1 - e^{-b_j t}\right) \right], \quad (13)$$

where

$$b_j = k_{+1j} (S_j + k_{mj}) \quad (14)$$

and

$$A_m = \prod_{\substack{j=1 \\ j \neq m}}^{n} \frac{b_j}{b_j - b_m} \quad \left(\text{note } \sum_{m=1}^{n} A_m = 1 \right). \quad (15)$$

At first sight, this formula leaves nothing but a slight dizziness, at least with me, but when one becomes better acquainted with it, it allows exactly the same general conclusion as the simple one did: A cascade causes no amplification, but an avalanche or multiplier effect.

It is easily seen that in the formulae giving product formation as a function of time in open cascades, time figures elevated to the power n (n being the number of steps). Clotting time (t_c), which is essentially the time necessary for a certain amount of product to be formed, will therefore never be a simple function of the substrate concentrations and reaction constants of an open cascade.

The kinetics of a damped cascade

In a damped cascade each step of the process is represented by

$$S_1 \overset{k_{+1}}{\rightarrow} E_2 \overset{k_{+2}}{\rightarrow} I_2.$$

The simplest possible kinetics of this step occur when $v_{+1} = k_{+1} S_1$ and $v_{+2} = k_{+2} E_2$. This case has been treated by Levine (1966). Apart from an apparent printing error we come to the same conclusion as he did, i.e.

$$P_t = E_1 \prod_{j=1}^{n} \frac{S_j k_{+1j}}{k_{+2j}} \sum_{j=1}^{n} A_j (1 - e^{-k_{+2j} t}) \quad (16)$$

in which

$$A_j = \prod_{\substack{p=1 \\ j \neq p}}^{n} \frac{k_{+2j}}{k_{+2j} - k_{+2p}} \quad \left(\text{note } \sum_{j=1}^{n} A_j = 1 \right). \quad (17)$$

As t approaches infinity, e^{-kt} approaches zero. So with time the whole form will become independent of t, or in other words, the concentration of the final product will approach a constant level. This formula is only valid when the final product is broken down as well as all the intermediate products. From this formula another expression can be derived to cover the case in which the last product of a damped cascade is not broken down but accumulates. In that case, in an $n+1$ step cascade

$$v_{n+1} = k'_{n+1} S_{n+1} E_n. \quad (18)$$

Provided S_{n+1} is present in excess, as in the case, for instance, in blood coagulation experiments in which the concentration of fibrinogen is hardly ever rate determining, this simplifies to

$$v_{n+1} = k_{n+1} E_n \quad (k_{n+1} \neq k'_{n+1}) \quad (19)$$

Application to the study of blood coagulation

If you will return with me now to the laboratory, I will show you some experiments concerning a complete cascade in which the concentration of each of the reactants was varied separately. The cascade under study is the extrinsic blood coagulation system. This cascade consists of the proenzymes factor VII, factor X, factor V, and factor II, and further needs phospholipids, Ca^{2+} ions and a still poorly defined protein from tissue ('tissue factor'). The substrate of the last step is fibrinogen, the product is fibrin (for full experimental details see Hemker & Muller 1968). The over-all reaction velocity in such a system is assessed by measuring a clotting time; that is essentially the time necessary for a fixed amount of fibrinogen to be converted into fibrin. A practical difficulty arises in these systems from the circumstance that it is not possible to obtain plasmas that are completely devoid of one specific factor. This difficulty is demonstrated by the fact that in a reaction medium planned to be deficient in one factor, coagulation occurs when this factor is *not* added to the system if the system is provided only with tissue thromboplastin (i.e. protein factor from tissue and phospholipids) and Ca^{2+} ions. (The clotting time then obtained is the so-called buffer value, $t_{\text{buff.}}$). The cause of this reaction can be either that a small percentage of the factor planned to be absent still is present or that another plasma protein functions as a stand-in for the deficient factor. In the latter case the functional amount of the deficient activity that is still present can be expressed as an equivalent concentration of the normal factor. A fundamentally different explanation of the observation that a deficient plasma still clots would be that a different reaction pathway takes over—one in which the deficient factor is not required. A choice between these two possibilities can be made on the basis of the following considerations:

(*a*) No alternative pathways are known when a system is deficient in one of the factors II, V, or X.

(*b*) (i) If an alternative pathway exists, it will be active when the deficient factor is added but will be overshadowed by the activity of the main pathway. The overall reaction velocity would then be a result of the combined activities of both pathways. The 'buffer time' is determined by the velocity of the alternative pathway only. The velocity in the pathway under study ($v_{\text{corr.}}$) can therefore be found by subtracting the velocity giving the buffer time ($v_{\text{buff.}}$) from the over-all velocity ($v_{\text{exp.}}$). If the coagulation velocity is defined as the inverse of coagulation time, this gives:

$$v_{\text{corr.}} = v_{\text{exp.}} - v_{\text{buff.}} = 1/t_c - 1/t_{\text{buff.}}. \tag{26}$$

The correlation between $v_{\text{corr.}}$ and the concentration of the factor (C) that is rate limiting in the pathway under study can then be determined experimentally. When $v_{\text{corr.}}$ of the extrinsic pathway was determined in this way, we found no comprehensible correlation between C and $v_{\text{corr.}}$ (Hemker *et al.* 1965*a*; Hemker, Van der Meer & Loeliger 1965*b*).

(ii) When, however, we assume that one pathway is operative but that, besides

the amount of the rate limiting factor added (C), a certain unknown amount (L) of the same factor was present in the reaction mixture, it will be clear that $v_{exp.}$ is determined by $(C+L)$, whereas $v_{buff.}$ if determined by L only. The nature of the relationship between v and the concentration of the rate limiting factor would then be essentially the same, whether $v_{exp.}$ is expressed as a function of $(C+L)$ or $v_{buff.}$ as a function of L. The latter approach proved to be the more fruitful one. It could be shown beyond all reasonable doubt that the inverse of the concentration $(C+L)$ against $1/v_{exp.}$ is a straight line (linear regression coefficient 0·998 in the case of factor II and factor VII determinations estimated from 500 experimental points each). The same linear relation between factor concentration and coagulation time was found for factor V and factor X. It was not found in the intrinsic system, nor was it found when factors VII and X were varied simultaneously. Factor I was shown to be present in excess (Hemker *et al.* 1965*a*).

This experimental outcome leaves us with the problem that in all cases in the extrinsic blood coagulation pathway the relation between reaction velocity and the concentration of the rate limiting coagulation factor is of the type

$$vp = \frac{kES}{K+S} \tag{27}$$

(p being an arbitrary constant).

Comparison with equation (2) shows that the over-all reaction velocity in the extrinsic cascade bears a relation to the concentration of each of its substrates that perfectly imitates the relation found in a simple system. On the basis of the theoretical considerations given above, we must conclude that it is likely that a damped cascade is operative in this system. This, in combination with the results of other experiments (Hemker, Esnouf, Hemker, Swart & Macfarlane 1967), led us to postulate the following reaction mechanism, which you will recognize as essentially the same as that proposed by Dr M. P. Esnouf (this volume, p. 269):

$$\mathrm{X} \xrightarrow{\mathrm{VII_a}} 2\mathrm{Xa},$$

$$\mathrm{Xa} + \mathrm{V} + \text{phospholipid} \rightleftarrows \text{prothrombinase} \rightarrow \text{inactive prothrombinase},$$

$$\mathrm{II} \xrightarrow{\text{prothrombinase}} \text{thrombin} \xrightarrow{\text{antithrombins}} \text{inactive thrombin},$$

$$\text{fibrinogen} \xrightarrow{\text{thrombin}} \text{fibrin}$$

The interaction of fibrinogen, thrombin, and fibrin shows some interesting features which cannot be discussed here. For a more detailed discussion, see Hemker & Muller (1968).

References (Hemker & Hemker)

Dixon, M. & Webb, E. 1965 *Enzymes*, 2nd edn. London: Longmans Green and Co.

Gutfreund, H. 1965 *An introduction to the study of enzymes*. Oxford: Blackwell Scientific Publications.

Hemker, H. C. 1965 In discussion to Macfarlane, R. G. *Thromb. Diath. Haemorrh. Suppl.* **17**, 63.

Hemker, H. C., Esnouf, M. P., Hemker, P. W., Swart, A. C. W. & Macfarlane, R. G. 1967 *Nature, Lond.* **215**, 248.

Hemker, H. C., Hemker, P. W. & Loeliger, E. A. 1965*a* *Thromb. Diath. Haemorrh.* **13**, 155.

Hemker, H. C., van der Meer, J. & Loeliger, E. A. 1965*b* *Thromb. Diath. Haemorrh. Suppl.* **17**, 247.

Hemker, H. C. & Muller, A. D. 1968 *Thromb. Diath. Haemorrh.* **20**, 78.