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Developing the enzyme-machine analogy: a non-mathematical approach to teaching Michaelis-Menten kinetics

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ABSTRACT: *The behavior of enzyme-catalyzed reactions is not made clear to many students by the standard mathematical description of enzyme kinetics. An enzyme-machine analogy is described that has made the details of the Michaelis-Menten mechanism and the associated kinetics more accessible with minimal use of mathematics. Students taught using the analogy appear to have fewer of the misconceptions than those taught using a more mathematical approach.*

Keywords*: analogy; enzyme kinetics; machine; teaching*

Introduction

Enzymes are often described as molecular machines [1], for example the F_oF_1-ATP synthase has been compared to a Wankel rotary engine [2] and other enzymes are also reminiscent of machines [3]. The actual and potential nanobiotechnological applications of biomacromolecules simply reinforce the idea that the enzyme-machine (E-M) analogy is more than just a metaphor [4].

However, analogies must be developed and used carefully because they can engender significant misconceptions [5]. Orgill and Bodner [6] suggested that good analogies are simple, easy to remember and based on familiar analogue concepts, and that they should be used on the introduction of a difficult or challenging concept that cannot be visualized, but not when the target concept is overwhelming or has to be memorized. To be most effective, the elements of an analogy must be made clear and its

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limitations need to be explained.

Students find enzyme kinetics a challenging topic. This is unsurprising given that the concepts are expressed mathematically [7,8] and rely on several ideas that are particularly difficult for students (such as the mole concept and kinetic theory [9]), and the molecular processes can not be visualized directly. However, it is much less daunting if the student *understands* and does not feel the need to *remember*. The E-M analogy is simple, based on a familiar analogue concept, and helps to promote student comprehension and minimizes the pressure to memorize. Moreover, it can be used to link enzyme kinetics with other aspects of protein function, such as ligand binding and regulation. While the E-M analogy has been used before [10,11], it has yet to be fully developed, as I do here.

The Michaelis-Menten mechanism for the conversion of S ([S] = s) to P is

$$\mathrm{S} + \mathrm{E} \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} \mathrm{ES} \xrightarrow{k_{\mathrm{cat}}} \mathrm{E} + \mathrm{P} \qquad (1)$$

and the standard equations derived from it [12] are $v = k_{\mathrm{cat}}c = V_{\mathrm{max}}s/(K_{\mathrm{m}} + s)$, where $K_{\mathrm{m}} = (k_{-1} + k_{\mathrm{cat}})/k_1$ is the Michaelis constant and the maximum rate of reaction is $V_{\mathrm{max}} = k_{\mathrm{cat}}e_{\mathrm{t}}$, where e_{t} is the total enzyme concentration (= $e + c$, where e and c are the concentrations of E and ES, respectively). These equations appear to be simple, but many students struggle to see their implications. This is a result of the intrinsic challenges of chemistry [9] and the limited mathematical skill and confidence of many biology students [7,8], which reflects a more general decline in mathematical literacy [13-16]. For example, many students incorrectly define K_{m} as $\frac{1}{2}V_{\mathrm{max}}$, when it is 'obvious' that K_{m} must have units of concentration in order to be able to add s and K_{m}. Moreover, it follows from the Michaelis-Menten equation that if $s = K_{\mathrm{m}}$, then $v = \frac{1}{2}V_{\mathrm{max}}$. Anyone capable of this basic mathematical analysis should not make the mistake of defining K_{m} as $\frac{1}{2}V_{\mathrm{max}}$, but it may be easy to make this error if the relationship has to be memorized.

Such difficulties prompted me to develop the machine analogy to provide a more intuitive way of teaching basic Michaelis-Menten kinetics that does not rely on understanding the underlying mathematics, but helps many students. While this is clearly not an original idea, I have not seen it developed, as it is here, to include some features of enzyme kinetics that many students find relatively mysterious, while limiting the development of misconceptions.

Premises of the enzyme-machine analogy

Very many biochemistry teachers will have suggested that an enzyme is similar to a machine, because it brings the protein into a realm with which students are familiar and because machine metaphors are common in biology [17]. Here, the analogy is based

on the following premises:

a. A single enzyme corresponds to one machine and several enzymes are analogous to a factory containing the corresponding number of identical machines.

b. Each enzyme-machine (E-M) repeatedly converts a specific S into a specific P in the same way, and at the end of the cycle is ready to carry out the task again.

c. There are two warehouses in which are stored the raw material (substrate or S) and finished product (product or P), corresponding to the medium in which the reaction takes place.

d. The S nearest the E-M is removed from the warehouse before the S that is further away, but the S is equidistant from each E-M. A high concentration of S (*s*) is taken to be related to a smaller distance between S and the E-M.

e. The P is instantly removed from the vicinity of the E-M to prevent its accumulation interfering with further processing of S to P.

This model (summarized in table 1) illustrates several properties of the Michaelis-Menten mechanism and can be extended easily to incorporate concepts such as channeling.

Implications of the enzyme-machine analogy

1. The enzyme is a catalyst. In general, machines carry out tasks that a person could do given enough skill, time, energy and raw materials, but the machine does so much more rapidly and is not altered in the process. Similarly, an enzyme carries out a reaction that could, in principle, happen in its absence, but it does so much more quickly than would be the case in its absence. For example, ATP hydrolyses very slowly in solution ($k < 10^{-3}$ s^{-1}, [18]), but in the presence of F_1-ATP synthase the rate increases at least 10^5-fold ($k > 800$ s^{-1}, [19]), similarly, in the absence of carbonic anhydrase, the hydration of CO_2 is very slow ($k = 0.0375$ s^{-1}, [20,21]), but in the presence of the enzyme the reaction is accelerated 10^7-fold ($k = 8.1 \times 10^5$ s^{-1}, [22]). Neither enzyme is changed by the reaction catalyzed.

2. An enzyme has a specific catalytic mechanism. Like any machine, an enzyme carries out the same process repeatedly (unless something unusual happens). The mechanism (summarized by the Michaelis-Menten model) involves (i) the raw material being transported to the E-M, (ii) loading the E-M with raw material ($S + E \rightarrow ES$), (iii) the release of raw material from the E-M ($ES \rightarrow E + S$), and (iv) the conversion of the raw material into product and its release from the E-M ($ES \rightarrow E + P$). Naturally, phase (iv) is likely to involve several steps even for a simple manufacturing process, and, similarly, the enzymatic step summarizes some mechanochemical steps (Segel [23]

provides a comprehensive collection of various models, including these).

Table 1: Summary of the enzyme-machine analogy.

Characteristic	Enzyme	Machine
catalytic action	• accelerates reactions • not changed by the reaction	• generally accelerates a process that could be done manually given enough time, skill, energy and resources • at the end of the cycle it is returned to the initial state
specific mechanism	• $S + E \leftrightarrow ES \rightarrow E + P$	• repeated cycle of movements or processes
conservation of materials	• $e_t = e + c$ • $s_0 = s + c + p$	• number of machines in the factory does not change • stores of raw materials, products and material being processed are constant
activity increases with the supply of raw materials	• $v = V_{max} s/(s + K_m)$	• the more materials, the shorter time a machine has to wait between cycles, but an upper limit is determined by the processing time
activity rises with the number of E-Ms	• $V_{max} = k_{cat} e_t$	• the more machines, the greater the productivity
V_{max} is approached asymptotically	• $c/e_t = s/(s + K_m) < 1$	• a machine must be free of materials before more can be loaded, so there is always a free machine, on average
K_m is independent of E-M number	• $K_m = (k_{-1} + k_{cat})/k_1$	• the K_m is a property of each machine, so the factory has the same K_m
K_m reflects the affinity of E-M for S	• $K_m = (k_{-1}/k_1) + (k_{cat}/k_1)$ $= (K_1)^{-1} + (k_{cat}/k_1)$	• if the machine tends to load raw material more often than it unloads it, then the proportion of time spent processing raw materials is greater
K_m is inversely related to the efficiency of E-M	• $\phi = k_{cat}/K_m$ is the first order rate constant for the reaction ($v/e_t \approx \phi s$)	• as K_m increases, the proportion of time a machine spends processing materials decreases
inhibition	• inhibitors bind to E, ES or either, with different effects on K_m and V_{max}	• a machine can be slowed by loading the wrong material and/or by loading material when the machine is operating
channeling	• in multienzyme complexes, P is 'fed' directly to the next E	• in a factory with a production line, the product of one machine is passed to the next in the line

In solution, the frequency with which S encounters E depends on their proximity, which is related to the concentration of each, and on their interactions with the solvent and each other [24]. In the machine analogy, the frequency with which the raw materials are transported to the machine depends on the distance between S and each machine: the smaller the distance (corresponding to a greater concentration), the more rapidly the machine can be supplied with input.

On average, the binding of S to E to form ES takes a certain amount of time (the

average lifetime of E is $\tau_E = (k_1 s)^{-1}$ [25]) and the degradation of ES (ES $\rightarrow$ E + S and ES $\rightarrow$ E + P) takes another set period of time (the average lifetime of ES is $\tau_{ES} = (k_{-1} + k_{cat})^{-1}$ [25]). However, S may be released from ES as well as converted to P. The probability of the release of S (ES $\rightarrow$ E + S) rather than P (ES $\rightarrow$ E + P) depends on the relative sizes of k_{-1} and k_{cat}, respectively.

Of course, this model is an idealization in at least two respects. First, some enzymes do not carry out the same process every catalytic cycle either because of the possibility of binding different substrates (for example, ribulose 1,5-bisphosphate carboxylase/oxygenase can either carboxylate or oxygenate ribulose 1,5-bisphosphate yielding different products [26] and cytochrome P450s also utilize different substrates [27]). Second, even those enzymes that catalyze a single reaction need not do so at the same rate because the proteins are in various states [28,29] or because the kinetics can be modified by environmental conditions [30]. However, these phenomena might also be found in some machines. As a machine ages, it may not behave as it did when new and, even with maintenance, will have to be replaced eventually.

If the assumption of identical machines is relaxed, temporarily, to allow for mechanical failure, repair and, ultimately, replacement, then it is reasonable that some E-Ms will be replaced or have been serviced more recently than others. This implies that there might well be a range of values of k_1, k_{-1} and k_{cat}, and therefore of K_m and V_{max}, just because of the inevitable aging of each E-M.

3. Conservation of materials *($e_t = e + c$ and $s_0 = s + p + c$).* Unless some of the machines are removed (for repair, for example) then the total number of machines in the factory remains the same, even if they are in different states (E rather than ES, for example). Similarly, unless some of the P is dispatched from the warehouse, the total amount of materials ($= s + p + c$) cannot change. In biotechnological applications, the enzymes might be bound to a resin in a column and the substrate loaded onto the column and product removed from the bottom of the column, in which case the material is not conserved in the reaction volume.

4. Activity *(v and V_{max}) increases with e_t and s.* The more E-Ms there are operating in the factory, the more rapidly will P accumulate in the warehouse. Conversely, if some of the E-Ms are removed or incapacitated (by inhibition or from disrepair), the productivity (the product accumulated by the factory per unit time or v) will decline. This is consistent with the reduction in v associated with inhibition which is considered below.

If the E-M does not have to wait for the raw material (S) to be delivered, then the process can be carried out more frequently than if the E-M has to wait for raw materials. Put another way, the shorter the waiting time of each E-M for the input of raw materials, the closer to maximum productivity will the factory operate. Since the transfer of raw materials to the E-M relates to the distance between S and the enzyme, which corresponds to *s* (as described above), up to a certain point, the greater the stockpile of raw materials, the less time any machine will have to wait for its supply. Implicit in this idea are two other concepts:

a. the speed of processing is independent of *s* (that is, the catalytic step takes a particular time once S is bound to the enzyme, as discussed above), and

b. V_{max} is approached when the supply of S is no longer limiting, rather this is a limitation arising from the supply of unoccupied enzyme (that is E rather than ES), a point that will be considered further below.

5. Maximum activity *(V_{max}) is a limit rather than a rate that can be achieved.* An E-M cannot start another cycle (bind S) until P has been released because S binds to the same site from which P is released. This means that there must be an empty active site at one point during each catalytic cycle otherwise a new S cannot bind to the enzyme (this is explicit in the Michaelis-Menten mechanism: S + E $\rightarrow$ ES). No matter how rapidly the E-M carries out the task, this must be true, which means that the probability of an E-M being in the ES state is less than 1 ($\tau_{ES}/(\tau_E + \tau_{ES}) = s/(s + K_m) < 1$).

6. The K_m is independent of e_t. If there are no raw materials ($s = 0$), then no E-M will be occupied in converting S to P ($c = 0$ and $e = e_t$), but as the supply increases, so too does the proportion of time that the E-M can be occupied ($c \rightarrow e_t$ and so $e \rightarrow 0$). Somewhere between these two extremes, there is a particular s ($= K_m$) at which each E-M will be occupied (or unoccupied) half of the time (so $c = e = \frac{1}{2}e_t$), so each E-M will be converting S to P at half of the maximum rate ($v = \frac{1}{2}V_{max}$). If $s = K_m$, $k_1K_m = (k_{-1} + k_{cat})$ and $\tau_E = \tau_{ES}$, so the E-M is occupied (or unoccupied) half of the time.

Providing that raw materials are supplied to a machine at an appropriate rate (corresponding to an appropriate *s*, as described above, equal to K_m), it will be occupied only half of the time (that is, 50% of the time it will be involved in converting S to P). This rate of supply depends on the length of time the E-M is involved in the conversion of S to P (τ_{ES}), rather than the number of E-Ms (e_t). The same is true for each machine in a factory of identical machines, the longer the catalytic cycle takes, the fewer raw materials required. In a factory of identical machines, the same can be said for each machine, so the K_m is independent of e_t.

Even if this argument does not help some students, most will accept that K_m is a

property of each individual E-M, since it depends only on the three rate constants (k_1, k_{-1} and k_{cat}). Subject to the possibility of E-Ms of different ages, every E-M has the same K_m and so does the population, irrespective of how many there might be.

The K_m is often said to be related to the affinity of E for S, by which is meant the tightness of the binding of S by E [31,32], and to the efficiency ($\phi = k_{cat}/K_m$) of the enzyme [33]. Strictly, K_m reflects the affinity of E for S when $k_{-1} >> k_{cat}$ (in which case $K_m \approx k_{-1}/k_1$), otherwise the interpretation is more complicated. The link with affinity is based on the first (reversible) step of the Michaelis-Menten mechanism which relates to S binding (equation (1)) and has an equilibrium constant $K_1 = k_1/k_{-1}$ (for the reaction as written in equation (1)). The larger K_1, the greater is c/e for a given s, which implies that E binds S more 'tightly' than would be the case if K_1 were smaller, equivalently, the higher the frequency of rejection (k_{-1} compared with k_1), the lower the affinity of the machine for raw materials. Since $K_m = (k_{-1} + k_{cat})/k_1 = K_1^{-1} + k_{cat}/k_1$, a large K_1 corresponds to a smaller K_m and a smaller s required to keep E occupied half of the time. Obviously, if a machine rarely unloads the raw material, then that raw material is bound tightly or with relatively high affinity.

7. Inhibition is the specific inactivation of enzymes. A machine may cease to operate if (i) defective or inappropriate raw materials are loaded, (ii) an attempt is made to load material before it is ready or (iii) either of these. The first case corresponds to competitive inhibition in which an inhibitory compound (I) binds to E (but not to ES). The greater the proportion of normal raw materials (S) to defective materials (I), the more frequently the E-M will carry out a normal cycle (V_{max} is not affected by I) , but the presence of I decreases the efficiency with which the E-M is loaded, increasing K_m. The second case corresponds to noncompetitive inhibition in which I binds to ES (but not to E). Since the E-M binds the raw material normally, K_m is unaffected, but an E-M to which I is bound is inactive and increasing the supply of S does not overcome this effect (so V_{max} is reduced). The third case is simply a combination of the first two possibilities so I binds to either E or ES (so it is known as mixed inhibition).

Conclusion

Biochemistry, like chemistry and physiology, is a demanding discipline [9, 34, 35] and, partly because much of it deals with what cannot be seen directly, is rich in analogies. Students find enzyme kinetics challenging, which is unsurprising given that it is based on several of the concepts that Sirhan [9] identified as particular sources of difficulty for students (for example the mole concept, kinetic theory, thermodynamics and intermolecular forces), involves mathematics [7,8] and the molecular processes can not be visualized directly (for example, even where it is possible to 'watch' a single molecule in operation, the molecular processes involved must be inferred from

experiment [36]). This has necessitated the development of analogies [37-39] to assist students to comprehend molecular processes.

The E-M analogy outlined here (summarized in Table 1) is simple, easy to remember and based on a familiar analogue concept. The analogy is developed here more fully than it has previously been. Moreover, it is effective in that it helps students *understand* the concepts rather than having to *remember* them. For example, it almost eliminated the K_m = ½V_{max} misconception.

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References and Notes

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