Breaking Bonds versus Chopping Heads: The Enzyme as Butcher

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Breaking Bonds versus Chopping Heads
The Enzyme as Butcher

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Enzyme catalysis is a core topic in any introductory biochemistry course. It is sometimes difficult for students to relate kinetic parameters from the Michaelis–Menten treatment to the actual behavior of the enzyme-substrate complex. Intricate aspects of enzyme activity such as allosteric regulation and cooperativity are even harder to assimilate. I have found that likening an enzyme to an old-fashioned butcher (or fowl slaughterer) helps students visualize many of these abstract concepts and invariably makes them smile as well!

Michaelis–Menten Kinetics

In the classical Michaelis–Menten scheme, the enzyme (E) binds substrate (S), forming a non-covalent enzyme-substrate complex (ES) which lowers the activation energy for converting substrate to product (P):

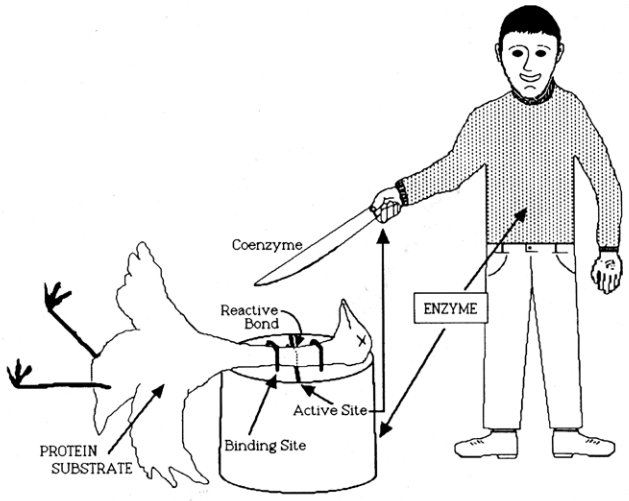
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} E{\cdot}S \xrightarrow{k_2} E + P \qquad (1)$$

From this scheme the initial reaction rate (v_o) can be shown to be

$$v_0 = \frac{k_2[E]_0\,[S]_0}{K_m + [S]_0} = \frac{V_{max}}{1 + K_m\,/[S]_0} \qquad (2)$$

where $V_{max} = k_2[E]_o$ = the maximum (initial) rate approached as $[S]_o$ is increased, and $K_m = (k_{-1} + k_2)/k_1$ = the Michaelis–Menten constant. Under normal conditions, substrate binding is very fast relative to bond-breaking ($k_1 \approx k_{-1} >> k_2$), so the Michaelis–Menten constant is

$$K_m = \frac{(k_{-1} + k_2)}{k_1} \approx \frac{k_{-1}}{k_1} = K_d \qquad (3)$$



the dissociation constant for the ES complex. While not all enzyme catalytic processes are as simple as that specified above (i.e., single substrate, single catalytic step), for any enzymatic process we can safely assume that V_{max} will be influenced only by enzyme concentration and by the rate constant (k_{cat}) of the rate-determining step:

$$V_{max} = k_{cat}[E]_o \qquad (4)$$

(For the simple two step reaction specified in eq (1), $k_{cat} = k_2$.) Hence, the two parameters, k_{cat} and K_m, give insight into interactions at the enzyme's active (catalytic) site and at its substrate binding site, respectively.

Active Site (k_{cat}) versus Binding Site (K_m)

As depicted in the accompanying diagram, the butcher with his knife and chopping block represent the enzyme, while the goose whose neck is about to be cut represents the substrate. The butcher steadies the goose on a specially designed chopping block (binding site) that orients the neck (labile bond) optimally for chopping (bond cleavage). The enzyme/butcher uses a specially sharpened knife (a critical nucleophile or coenzyme) for cutting. The active

site consists of the butcher's arm and knife, poised over the fowl's neck. The binding site on the chopping block holds the animal (substrate).

The butchering analogy stresses that while binding interactions optimize orientation effects, active site interactions optimize bond breaking/reforming processes. In this way, we see that the overall reaction rate, v_o, is dependent on both optimal substrate binding (low K_m) and effective active site catalysis (high k_{cat}). This can be seen mathematically in eq (2).

Activation Energy (E_a)

Enzyme-substrate interactions serve to both stabilize the transition state (goose with neck half cut) and to destabilize the substrate (goose), thus lowering the activation energy and allowing the reaction to run faster. In the absence of the catalytic assembly (chopping block and poised knife), the butcher would be reduced to chasing the goose around the yard while wildly swinging a dull knife –a very slow and inefficient process!

Substrate Saturation

A key aspect of enzyme kinetics is substrate saturation. A single butcher can kill only one goose at a time, and the maximum butchering rate depends on how fast he can grab a goose, bind it to the block, lower the knife, and release the butchered animal. Let's assume that grabbing takes nine minutes, and binding, chopping, and releasing take only one minute. The chopping block is only occupied 10% of the time. By doubling the number of fowl in the yard we cut the grabbing time to 4.5 min, and the block is occupied 18% of the time. We have decreased the turnover time from 10 min to 5.5 min, nearly doubling the reaction rate.

At a certain point, however, with thousands of geese running around in the yard (excess substrate), the grabbing time will be reduced to just a few seconds. Binding/chopping/releasing still take one minute, so the block is almost always occupied. Further increases in fowl concentration do not significantly decrease the turnover time because it is already very close to its minimum of one minute. Hence, the reaction rate, v_o, asymptoptically approaches a maximum, V_{max}, when substrate is present in excess.

Rate Dependence on Enzyme Concentration

Adding a second independent butcher under these conditions will double the reaction rate, because in 10 minutes two geese can be butchered instead of one. Here we see that reaction rate is proportional to enzyme concentration.

Cooperativity

Students very often have a difficult time understanding why increased cooperativity in an allosterically regulated enzyme has an inhibitory effect on the enzyme's activity. The butcher analogy provides a convenient way to think about this problem.

What happens if we force two butchers to behave cooperatively, that is, they can only chop if geese are bound on both blocks. Under comparable non-saturating conditions (nine minutes to grab, one minute to bind/chop/release) it will take more than nine minutes to bind a goose at both blocks. In a typical cooperative interaction, binding of the first goose makes binding of the second one easier (via various allosteric effects). For example, if it takes nine minutes to bind the first goose, it might take only three minutes to bind the second one. Finally, it will take one more minute to slaughter both, for a total elapsed time of 13 minutes. If the two butchers had been acting independently (non-cooperatively), then the two geese would have been slaughtered in only 10 minutes. So cooperativity has an inhibitory effect on reaction rate.

The key here is that cooperative sites bind substrate sequentially. This takes longer than at non-cooperative sites, which can each bind substrate simultaneously and independently of each other. This is an important point that often gets lost in the discussion of cooperativity and enzyme inhibition. When students approach this from the butchering analogy, they often have one those "Aha, now I understand!" experiences. Similar student breakthroughs in understanding the effects of enzyme inhibitors also are attainable, as outlined below.

Allosteric Effects

The inhibitory effect of enhanced cooperativity discussed above is an allosteric effect. Binding events at one chopping block influence events at another, physically separate block. Other types of allosteric inhibitory effects might involve the butcher's arthritic neck or shoulder slowing down his hatchet arm's chopping rate. Alternatively, a chip on the bottom of the chopping block could make it unsteady enough to inhibit binding or chopping.

Irreversible Inhibitors

Irreversible inhibitors permanently poison the enzyme catalyst. If the knife blade is broken or removed, k_{cat} falls permanently to zero. If the block's binding site is radically deformed, K_m increases to infinity (no geese bound). Suicide substrates actually bond permanently to the enzyme's active site or binding site: a goose with super glue on its throat would stick to the knife, clogging the active site; a goose with super glue on the back of its head would stick to the block, clogging the binding site.

Reversible Inhibitors

Reversible inhibitors partially reduce the reaction rate, and this effect can be reversed by removing the inhibitor (as opposed to the permanent effects caused by irreversible inhibitors). Competitive inhibitors often bind at the binding site like a true substrate, but then don't undergo subsequent reaction. Consider a goose with a protective steel sleeve around its neck: this bum fowl occupies the active site, but doesn't get slaughtered. It "wastes" 10 min of the butcher's time, slowing his slaughtering rate. As excess normal geese are added, the butcher's chances of catching a bum fowl approach zero, and v_o approaches the same V_{max} as in the absence of competitive inhibitor. The overall efficiency of binding normal geese is compromised, however, so the "apparent" K_m increases.

Finally, non-competitive inhibitors bind to the enzyme-substrate complex and decrease k_{cat}. Anything hindering the butcher's swing (like arthritis), his blade's sharpness, or the exposure of the goose's neck to the blade would tend to decrease the reaction rate. Because these effects are totally independent of binding, K_m remains unchanged, but k_{cat} decreases. Note that even in the presence of excess geese, v_o approaches a V_{max}' value that is lower than that found in the absence of the non-competitive inhibitor. An arthritic butcher with a dull knife will always be slower than an unhindered butcher with a sharp knife, no matter how many geese are available.

Of course an arthritic butcher with a dull knife would quickly lose his butchering license, but here I suppose the analogy must end.