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Nonlinear Enzyme Kinetics Can Lead to High Metabolic Flux Control Coefficients: Implications for the Evolution of Dominance*

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In a classic study, Kacser & Burns (1981, *Genetics* **97,** 639–666) demonstrated that given certain plausible assumptions, the flux in a metabolic pathway was more or less indifferent to the activity of any of the enzymes in the pathway taken singly. It was inferred from this that the observed dominance of most wild-type alleles with respect to loss-of-function mutations did not require an adaptive, meaning selectionist, explanation. Cornish-Bowden (1987, *J. theor. Biol.* **125,** 333–338) showed that the Kacser-Burns inference was not valid when substrate concentrations were large relative to the relevant Michaelis constants. We find that in a randomly constructed functional pathway, even when substrate levels are small, one can expect high values of control coefficients for metabolic flux in the presence of significant nonlinearities as exemplified by enzymes with Hill coefficients ranging from two to six, or by the existence of oscillatory loops. Under these conditions the flux can be quite sensitive to changes in enzyme activity as might be caused by inactivating one of the two alleles in a diploid. Therefore, the phenomenon of dominance cannot be a trivial "default" consequence of physiology but must be intimately linked to the manner in which metabolic networks have been moulded by natural selection.

Introduction

Few issues in genetics have been as contentious as the basis of dominance and recessiveness (Nanjundiah, 1994). A major step towards understanding the problem, and a plausible solution, was provided by Kacser & Burns (1981). The solution emerged through a study of how flux is regulated in metabolic pathways (Kacser & Burns, 1973, 1981; Heinrich & Rappoport, 1974). It was shown that when an unbranched pathway is made up of large number of linear (i.e. Michaelis–Menten) enzyme-catalysed reactions, all operating well below saturation, the overall flux at steady state is insensitive to the level of any single enzyme in the pathway. Mathematically, this can be expressed as follows in terms of the sensitivity theorem. Let J be the steady-state flux and e the concentration (more properly, activity) of any enzyme. Then the flux control coefficient C, defined as $\partial \ln J/\partial \ln e$, and a measure of the sensitivity of J to changes in e, is of order $1/n$ where n is the number of reactions in the pathway. Therefore, for large n, $C \ll 1$. In other words J hardly changes at all when e is varied—short of falling to zero, at which point the pathway may no longer operate.

In relation to the problem of dominance and recessiveness, the sensitivity theorem shows that given the right conditions, when a gene codes for an enzyme, cellular physiology is indifferent to whether the number of copies of the gene is two or one. So a single dose of a gene should do as well as two and loss-of-function (null) mutations should be recessive to functional (wild-type) alleles. The implication is that in order to explain the observed dominance of the prevailing wild type to the overwhelming majority of mutant alleles, one needs to go no further than

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conventional biochemistry. Put differently, one does not need to invoke natural selection and seek an adaptive explanation for the dominance of the wild type—as one might want to in thinking of other properties of genetic systems, for instance. A purely physiological explanation for dominance ought to be the hypothesis of choice. Apart from the obvious restriction to genes that encode enzymes and to pathways involving a large number of steps, this inference depends on the assumptions made in deriving the sensitivity theorem. The chief among these are that the relevant enzymes (i) operate below saturation, (ii) obey linear (Michaelis–Menten) kinetics, (iii) are not subject to feedback regulation and (iv) constitute a pathway that is functioning at a steady state. In principle, a violation of any of these conditions could invalidate the conclusion. For example, most enzymes do operate below saturation (equivalent to the familiar $S \ll K_{0.5}$ assumption relating the free substrate level to the level needed for half-maximal reaction velocity). But this begs the question of why they do so; the answer, as Cornish-Bowden (1987) points out, must be "... a consequence of selection, not mathematics". Be that as it may, a conventional point of view would dictate accepting (i) and (iv) as plausible assumptions. That leaves us with the task of examining what happens to the sensitivity theorem when enzymes obey nonlinear kinetics and feed-backs are permitted. As we will show, high sensitivities can result even when substrate concentrations are low, warranting a second look at evolutionary explanations for dominance.

Model

GENERAL SCHEME

We consider a reversible pathway of the form

$$S(1) \rightleftarrows S(2) \rightleftarrows \ldots. \rightleftarrows S(n) \rightleftarrows S(n+1)$$

where $S(i)$ is the substrate in reaction i catalysed by enzyme E(i), and there are 20 enzymatic steps in all ($n = 21$). Each enzyme is characterised by the parameters $V_b(i)$, $V_f(i)$ (standing for backward and forward maximal velocity respectively), $M_b(i)$, $M_f(i)$ (the corresponding "Michaelis" constants) and $K(i)$, the equilibrium constant. (The quotation marks around "Michaelis" are as a reminder that in the case of a nonlinear enzyme these are not true Michaelis constants.) Only four of these five parameters can be chosen independently; the Haldane relation demands that $K = M_b V_f / M_f V_b$ for every i. The levels of $S(1)$ and $S(n+1)$ are held fixed. At steady state, the flux through the pathway, J, is the same as the velocity of any reaction step, which for the step from $S(i-1)$ to Si) is given by

$$J = \frac{V_f(i)\left[S(i-1) - \dfrac{S(i)}{K(i)}\right]}{M_f(i)\left[1 + \dfrac{S(i)}{M_b(i)}\right] + S(i-1)}$$

when all the enzymes are of the Michaelis–Menten type. Cooperativity, or nonlinearity, is modelled by the mathematically justifiable approximation of raising the relevant S(i) to the power $h(i)$, where $h(i)$ is a Hill coefficient.

The effect of feedback is considered separately by inserting a biochemical oscillatory mechanism within the pathway. The oscillator, consisting of three components α, β and γ, was borrowed from the scheme advanced by Goldbeter & Segel (1977) for cyclic AMP oscillations in the cellular slime mold *Dictyostelium*. (β is a product of the hydrolysis of α and the $\alpha \rightarrow \beta$ reaction is catalysed by an allosteric enzyme which is subject to a high degree of activation by γ.) It was inserted as a "box" between substrates $S(18)$, the input into the oscillator, and $S(19)$, the output. In relation to the rest of the pathway, $v = k(1)S(18)$ is a constant input flux in the rate equation for α and $k(2)\gamma$ is a time-dependent output flux from the oscillator and at the same time an input term for the rate of change of $S(19)$. Other than this oscillatory "box", the rest of the pathway was exactly as described earlier except that in this case all the enzymes were Michaelian.

CHOICE OF PARAMETERS

The pathway consisted of 20 steps. We attempted to choose as unrestricted a set of parameter values as possible. The choices were limited by two considerations. One was that we wanted a net flux from left to right, that is, from $S(1)$ to $S(n+1)$. This could have been achieved by taking each $K(i) > 1$. However, we also wanted to consider a pathway in which the overall reaction could proceed in either direction, suggesting a choice of $K(i) = 1$. A compromise was reached by taking $V_f(i) > V_b(i)$ for each i. Our other consideration was that after allowing for the previous restriction, all the parameters of the pathway had to be chosen randomly: the point being that we wanted to monitor the control coefficient in a background of more-or-less total uncertainty with regard to the characteristics of the enzymes governing the individual steps. All K

values were put equal to 1. V_f values were assumed to be independently and uniformly distributed random numbers varying in the range 1 to 10 and V_b values varied similarly in the range 1 *to* V_f, so that $V_f > V_b$ for each enzyme separately. The M_f values were also chosen randomly in the range 1 to 10, and M_b values were calculated from the Haldane relationship. When nonlinearities were considered, h values were chosen to vary between two and six, also from a uniformly distributed set of random numbers. A range of initial concentrations $S(1)$ was examined, from $S(1) = 0.5$ (all enzymes below saturation) to $S(1) = 100$ (essentially all enzymes saturated). $S(21)$ was invariably taken as zero. In the event that an oscillatory step was interposed—as explained, this was between $S(18)$ and $S(19)$—all parameter values for the oscillatory intermediates were as in Goldbeter & Segel (1977) except for their "dilution factor" which we took equal to ten instead of five for reasons of computational convenience.

CALCULATIONS

Ideally, one would like to calculate a flux J from $S(1)$, $S(21)$ and the enzyme parameters. The algebraic manipulations required to do so were forbidding, and we adopted the following strategy instead. We assumed a value for J and $S(1)$ and calculated downstream, as it were, until $S(21)$ was reached. On occasion an intermediate $S(i)$ would have a negative value, implying that the initial value of J was too high to be sustained. When that happened J was lowered by a small amount and the exercise repeated. The procedure was continued until a value of $S(21) \approx 0$ was reached. The final value of J was taken to be the maximum flux that could be supported by the pathway (given the assumed $S(1)$, and $S(21) \approx 0$). Control coefficients were estimated by choosing an enzyme at random and "mutating" it by systematically varying its V_f (and so V_b as well) in small increments. By re-calculating J with these new values, we were able to derive a quasi-continuous relationship between J and enzyme activity. By repeating the procedure five times we were able to compare average values of J with values according to the "low substrate level" approximation in Kacser & Burns (1981). This was followed by a numerical calculation of the control coefficient C at the starting value of V_f. Twenty replicates of the entire process were run, the choice of which enzyme to mutate being made randomly and independently on each occasion. From the data we estimated a typical, or in a loose sense "average", control coefficient characterising the pathway as a whole.

When the oscillator was interposed between $S(18)$ and $S(19)$, a short cut was used. We started with an input flux that fell just short of permitting oscillatory behaviour and then increased this flux in small increments. The magnitude of the increments corresponded to that expected (on the basis of the previous calculation) from "mutating" an upstream enzyme.

Results

In what follows we use the short form K-B to stand for the Kacser & Burns (1981) publication and the conclusions that follow from the assumption that $S(i)$ is small compared with $M_f(i)$ and $M_b(i)$ for all i. In all cases uncertainties prefixed by the sign $\pm$ represent standard deviations.

(i) In the absence of cooperativity, and at low substrate concentrations [$S(1) = 0.5$], so that all enzymes work below saturation, the K-B result is quite robust. The computed flux is $89.6 \pm 0.4\%$ and the mean control coefficient is $99.7 \pm 3.1\%$ of that expected from K-B. Interestingly, C continues to behave as expected ($104.98 \pm 2.02\%$) when $S(1) = 75$, at which point most enzymes are expected to be saturated. Not surprisingly, at $S(1) = 75$, absolute fluxes are on average about 10% of the K-B expectation, i.e. much lower. Reducing the activity of an enzyme by one-half brings about a further decrease in the flux of around 5%. The outcome is different when the activities of many enzymes are lowered simultaneously. Even so, with ten out of 20 enzyme activities being reduced by one-half, the flux remains at $67.5 \pm 33\%$ of its starting value.

(ii) With cooperativity, the final outcome depends on both the level of saturation of an enzyme taken at random and on how many steps are cooperative. At low substrate levels [$S(1) = 0.5$] the flux decreases steadily with the number of steps that are cooperative; the control coefficient C on the other hand first increases and then decreases. When just two of the 20 steps in the pathway are catalysed by cooperative enzymes, the average value of C goes up almost three-fold, from 0.041 ± 0.023 to 0.116 ± 0.156. When all 20 steps are cooperative, C is 0.043 ± 0.080. Correspondingly, the average flux upon decreasing the activity of a single enzyme by one-half is 92% of its starting value and if the activities of any ten of the 20 enzymes are reduced simultaneously by one-half, the flux is 68% of the starting value, standard deviations being of the same order as in the absence of cooperativity.

(iii) Interposing an oscillator between substrates $S(18)$ and $S(19)$ has a striking effect on the output

flux, by which we mean in this case the time-averaged flux from $S(20)$ to $S(21)$. When the input flux to the oscillator is changed from 0.099 to 0.100, that is, by as little as 1%, the period of the oscillation changes by 17.4% and the output flux by 14.9%. A 3% increase in input flux, still much smaller than that expected under the best-case scenario if the activity of one of the upstream enzymes were to decrease by one-half, causes an 89% increase in period and a doubling of the output flux.

Discussion

We wanted to examine the consequences of reducing the activity of any one enzyme by one-half for a randomly constructed metabolic pathway; a pathway that was cobbled together, so to speak, rather than being the product of elaborate design. Equilibrium constants were taken to equal one so as not to bias the direction of equilibrium. Enzyme parameters were chosen randomly, once again with a view to eliminate the notion of design. Therefore, all our results refer to "average" expectations and this is reflected in the fact that actual outcomes vary from one simulation to the next. Obviously, in the case of a well-designed metabolic network, meaning one that has been appropriately moulded by selection, each of the perturbations to which we subject the pathway could well have had a far milder outcome than that we show in our results.

In the absence of nonlinear behaviour, the K-B result has an impressive range of validity [Results, (i)]. But in its presence, our findings reinforce the conclusion reached by Cornish-Bowden (1987): on the face of it, there is no reason to expect that control coefficients will be as low as those predicted by the model of Kacser & Burns (1981). This is so even when each enzyme in the pathway functions below saturation. [When $S(1) = 0.5$ and M_f is chosen to lie between one and ten, the maximum level of saturation $(S)/M_f)$ is 50% and on average 18 of 20 enzymes have more than three-fourths of their binding sites unoccupied.] Nonlinear enzyme behaviour, as reflected in a velocity vs. substrate relationship that deviates significantly from the Michaelian pattern, can cause the flux to become sensitive to enzyme activities. Admittedly our model for cooperativity assumes largish Hill coefficients (ranging from two to six), but they lie within the range of values reported in the literature. Cooperativity causes a relatively steep rise in enzyme activity as a function of substrate concentration at intermediate concentrations, whereas the activity is fairly constant at both low and high substrate levels. There can be a sensitive dependence of flux on enzyme activity when some steps, but not all, are catalysed by cooperative enzymes [Results, (ii)]. A different aspect of enzyme nonlinearity is the possible existence of feedback loops leading to oscillations in metabolite concentrations. This too can lead to drastic consequences for the control coefficient [Results, (iii)]. Small changes in input—indeed, far smaller than expected to occur when the activity of an enzyme is lowered by one-half—can lead to large, and potentially significant, changes in output. Both this conclusion and the one reached in the previous paragraph are based on the average outcome of mutating any enzyme in the pathway, not just a "nonlinear" enzyme.

In sum, it is advisable to be cautious in seeking to explain the origin of domnance from K-B. Dominance of the wild type can be expected when substrate levels are low, conceivably even when they are high, but not when enzymes exhibit significantly nonlinear behaviour. A selective explanation would then seem to be called for.

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