AN ENZYMATIC HORMESIS BOX

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Abstract. We present a simple enzymatic system that is capable of a biphasic response under competitive inhibition. This is arguably the simplest system that can be said to be hormetic.

Keywords: competitive inhibition, hormesis, Gröbner bases

1. Introduction

Though 21st century biology is excellent in collecting data, and very good at translating these data into therapeutical interventions, it is less good at identifying principles of biological organisation. It has long been suggested (see for example [3]) that hormesis is a principle of biological organisation. Whether it is or is not, which is a highly controversial matter as we discuss below, it would be useful to present simple mechanisms at any level of biological organisation that allow for hormesis. The present paper takes no position in the heated debate about hormesis and restricts itself to suggesting an enzymatic “hormesis box”, which might be of independent interest to students of enzymology.

2. An introduction to hormesis

To quote Wikipedia [9], Hormesis “is any process in a cell or organism that exhibits a biphasic response to exposure to increasing amounts of a substance or condition.”

We have chosen this definition as it is “ethically neutral”: it makes no claim about the substance or condition being beneficial or noxious (injurious).

A typical hormesis dose-response curve which is not so ethically neutral is shown in Figure 1. Here the agent is assumed to be injurious and by a hormetic response in such a situation one (contentiously) means a response in which an injurious agent in small concentration confers benefits on the organism; we chose to label the axes in this way to alert the reader to the crux of the controversy.

In Figure 1 LNT stands for “Linear-No-Threshold”, the basis of much health and safety legislation. If instead of “noxious” one puts “beneficial” (as in anti-cancer drugs), one should reflect the hormetic and the LNT responses around the abscissa, both, again, debatable statements.

To see the fervour with which the merits and demerits of hormesis as a general principle of organisation are discussed, consult, for example [7]. In our opinion, the clearest (though partisan) analysis of the difficulties with hormesis is in [8][Ch. 3]; that chapter is entitled “Hormesis Harms: the emperor has no biochemistry clothes”.

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Shrader-Frechette distinguishes three different assertions: [H], [HG] and [HD], and says that [H] is trivially true, [HG] is demonstrably false, and [HD] is ethically and scientifically questionable. Here

- **[H]**: (In a particular biological context) there exists an endpoint (an observable) for which a noxious agent exhibits a beneficial effect at low concentrations;
- **[HG]**: [H] is generalizable across different biological contexts, endpoints measured, and classes of chemicals;
- **[HD]**: [H] should be the default assumption in the risk-assessment [and hence in risk-regulation] processes.

We comment that methodologically it is not clear by what criteria [HG] can be derived from instances of [H]: what does “generalizable” really mean? and that [HD] cannot follow from [HG], no matter how much money it saves to a particular industry; LNT will always be much better equipped to withstand legal challenges.

However, the impetus for the present work is Shrader-Frechette's statement that [H] is “trivially true”. While Shrader-Frechette admits that [H] is well documented across plant, fungal and animal kingdoms, there must arise the question why this should be the case.

Our tentative answer is that hormesis must hitch a ride on some very general principle (or principles) of biological organisation and does not make this principle (or these principles) fitness-reducing.

### 3. A SIMPLE ENZYMATIC MODEL OF HORMESIS

We consider a system comprised of an enzyme $E$, a substrate $S$, a product $P$ and an inhibitor $I$. By hormesis in such a system we mean that the rate of production of $P$ should increase on adding
sufficiently small amounts of the inhibitor. Obviously, arbitrarily complex mechanisms leading to hormesis can be devised, involving, for example, additional transcription of genes encoding the enzyme $E$. We aim here for maximal simplicity and ask what frequently encountered mechanism can be superimposed on a single enzyme confronted with a (competitive) inhibitor so that the system is hormetic.

We start with the indisputable fact that many proteins exist in a dimeric form (there is even a special BTB “born-to bind” domain!) \cite{5}. The intuition behind the mechanism that we are proposing is as follows: in the absence of the inhibitor, most molecules of the enzyme $E$ are to be found in the inert, dimer form $E_2$. In addition to combining with the enzyme at its active site, the inhibitor also causes the dissociation of the dimer, this making more active enzyme available for the production of $P$.

Therefore the reactions we must consider are:

$$E + E \xrightleftharpoons[k_2]{k_1} E_2,$$  
$$E + I \xrightleftharpoons[k_4]{k_3} EI,$$  
$$E + S \xrightleftharpoons[k_6]{k_5} ES \xrightarrow{k_7} E + P,$$  
$$E_2 + I \xrightleftharpoons[k_9]{k_8} E_2I \xrightarrow{k_{10}} 2E + I.$$  

We assume that the substrate $S$ is in constant supply. We would like to understand under what conditions on the rate of production of $P$, i.e. the quasi-steady state concentration of the complex $ES$ increases as we add a small amount of the inhibitor.

Below we denote concentrations of species by square brackets enclosing the symbol of the species. The system (1) and the theory of enzyme kinetics \cite{1} implies that we need to find the expression for the concentration of $ES$ from the following system of two conservation laws for the total amount of the enzyme and the inhibitor,

\begin{align*}
[E_0] &= [E] + 2[E_2] + [EI] + [ES] + 2[E_2I], \\
[I_0] &= [I] + [EI] + [E_2I],
\end{align*}

and the four equations we get by making quasi-steady state assumptions for $ES$, $EI$ and $E_2I$:

\begin{align*}
k_3[E][I] &= k_4[EI], \\
k_5[E][S] &= (k_0 + k_7)[ES], \\
k_8[E_2][I] &= (k_9 + k_{10})[E_2I], \\
k_1[E]^2 &= 2k_2[E_2] + 2k_{10}[E_2I].
\end{align*}

Solving the system of equations (2)–(3) purely symbolically is a formidable task as the scheme (1) involves ten kinetic constants $k_1, \ldots, k_{10}$, and we also have to take into account stoichiometric constraints. Of course, 4 of the kinetic constants can be absorbed and non-dimensionalisation will get rid of one of the stoichiometric constraints. The remaining system will still have eight parameters, and as the goal of the paper is not an exhaustive analysis of (2)–(3), we make rather crude simplifications in order to exhibit the possibility of hormesis in this system. Intuitively, to get a hormetic response, we need both $k_1$ and $k_{10}$ to be “large”, so that without inhibitor most enzyme is in dimeric form $E_2$, and, once inhibitor is added, it causes enough release of the active enzyme $E$ from the pool sequestered in the dimer to raise the level of production of $P$. To exhibit
the relation between the strengths of the two processes that results in a hormonal response, we simply put
\[ k_2 = k_3 = \cdots = k_9 = 1, \]
and take \([E_0] = 1\), i.e. set the total concentration of enzyme equal to one, and assume that the concentration of the substrate \(S\) is constant, and satisfies \([S] \ll [E_0]\); in our computations below we use \([S] = 10\).

Thus we have a system of six equations in the variables \([E], [ES], [EI], [E_2], E_2I\) and \([I]\) with symbolic parameters \(k_1, k_{10}\) and \([I_0]\). Since
\[
\frac{d[P]}{dt} = k_7[ES],
\]
we are really only interested in \([ES]\) as a function of these parameters. Obtaining an explicit formula for \([ES]\) (satisfying \(0 < [ES] < [E_0] = 1\)), is still nontrivial, but a univariate polynomial satisfied by \([ES]\) with coefficients depending on \(k_1, k_{10}\) and \([I_0]\), \(P([ES], [I_0], k_1, k_{10})\) can be easily obtained in MAPLE using the Groebner package (it is denoted by poly in the code in Appendix A. For more information on Gröbner bases that have proved to be very useful in solving polynomial equations of enzyme kinetics, the reader is referred to [2].

Solving \(P([ES], [I_0], k_1, k_{10}) = 0\), we have that the rate of production of \(P\) in the absence of the inhibitor, i.e. with \([I_0] = 0\), which we denote by \(r_0\) is given by
\[
 r_0 = \frac{5(-3 + \sqrt{9 + k_1})}{k_1}.
\]

Now we use a regular perturbation expansion: we write
\[
[ES] = r_0 + r_1[I_0] + O([I_0]^2).
\]

Hence hormesis is equivalent to \(r_1 > 0\). Computing \(r_1\) shows that the condition for hormesis can be written in a very elegant way:

\[
(4) \quad k_1 > k_{1,\text{crit}} := \frac{7k_{10}^2 + 2k_{10} - 5}{(k_{10} - 1)^2}, \quad k_{10} > 1.
\]

From (4) it is clear that hormesis is not possible for any value of \(k_{10}\) is \(k_1 \leq 7\). In the simulation below we choose \(k_1 = 40, k_{10} = 50\) which clearly falls in the hormonal regime of (4), and from \(P([ES], [I_0], 40, 50) = 0\) find the unique value of \([ES]\) in \([0, 1]\) as a function of \([I_0]\). The results are presented in Figure 2 and clearly show biphasic response.

4. DISCUSSION

As observed in the Introduction, in Figure 1 the ordinate is in units of “benefit” to the organism as most emotionally charged discussions of ethics and philosophy of hormesis are couched in these terms. We do not commit to interpret the (counterintuitive) increase in the rate of production of \(P\) in our model system as the competitive inhibitor is added to a benefit, being entitled to do so by our ethically neutral definition of hormesis.

In this short paper we presented a plausibly general mechanism by which a hormonal biphasic response could be generated in a simple enzymatic system; what made the biphasic response possible was sequestration of most enzyme in a dimer and the inhibitor-regulated release of the monomers from the dimer. It would be interesting to know if systems where this mechanism is operational exist, and if they do, how frequent are they.

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The claim [HG] in ethically neutral terms states that biphasic effects are the norm. If true, this is in fact as astonishing claim as, in our context, it does not mention the inhibitor ever being encountered before in the history of a species; it could be a chemical that had been synthesized for the first time in history just before the exposure experiment.

Many of R. Rosen’s examples of anticipatory systems [6] can be explained away by recourse to an evolutionary argument: the system behaves in such and such a way (e.g. the product of the first in a chain of enzymatic reactions leads to an activation of transcription of the last enzyme in the chain) because such behaviour is “fitter” than its absence (as, in the above example, absence of such an “anticipatory” effect would lead to a buildup of an intermediate in the chain of reactions), in which case the description of such a system as “anticipatory” is redundant and has no explanatory power. Hence an ethically neutral form of [HG], if true, would indicate the existence of intrinsically anticipatory systems.

**APPENDIX A. MAPLE CODE FOR COMPUTATIONS USED IN THIS PAPER**

Here we show how to compute the polynomial in \([ES]\), \(poly\) and the relation between \(k_1\) and \(k_{10}\) (\(k_{1\text{crit}}\) below). Below \(ES\) corresponds to \([ES]_m\) etc.

```maple
with(Groebner):

# set E0 to 1 and S to 10
# we use I1 instead of I as I is reserved I Maple

E0:= 1: S := 10:
```
# conservation laws

e1:= E0-E-2*E2-EI-ES-2*E2I:
e2 := I0-EI-E2I-I1:

# set all constants equal to 1 apart from k1 and k10
k2:=1: k3:=1: k4:=1: k5:= 1: k6:=1: k7:=1: k8:= 1: k9:=1:

# QSSAs:
e3:= k3*E*I1-k4*EI:
e4 := -k7*ES-k6*ES+k5*E*S:
e5 := k8*E2*I1-k9*E2I-k10*E2I:
e6 := -k1*E^2+k2*2*E2+2*k10*E2I: # more terms here but all zero by QSSA.

F := [e1,e2,e3,e4,e5,e6];
poly:= Basis(F,plex(I1,E,E2,EI,E2I,ES))[1]:

# poly is a 5th order polynomial in ES with coefficients that depend on
# I0, k1 and k10. You need the solution of poly that lies between
# zero and E0.

ab := factor(subs(I0=0,poly));

r0:= solve(op(1,ab),ES)[1]:

# r0 is the value of ES when I0=0

ac := subs(ES=r0+r1*I0,aa):

# this is the regular perturbation expansion

ad:= coeff(ac,I0,1):

r1 := solve(ad,r1):

k1crit:= solve(r1,k1);

# gives the minimal value k1 must have as a function of k10 to give
# hormetic response
REFERENCES

[1] A. Cornish–Bowden, *Fundamentals of Enzyme Kinetics*, John Wiley and Sons, Weinheim 2013.
[2] D. Cox, J. Little, and D. O’Shea, *Ideals, Varieties, and Algorithms: an Introduction to Computational Algebraic Geometry and Commutative Algebra*, Springer, New York 2013.
[3] V. E. Forbes, Is hormesis an evolutionary expectation? Funct. Ecology 14 (2000), 14–24.
[4] Maplesoft, a division of Waterloo Maple Inc., *Maple*, Waterloo, Ontario 2019.
[5] R. Perez-Torrado, D. Yamada, and P.-A. Defossez, Born to bind: the BTB protein-protein interaction domain, Bioessays 28 (2006), 1194-1202.
[6] R. Rosen, *Anticipatory Systems*, Springer, New York 2012.
[7] B. Sacks, G. Meyerson, and J. A. Siegel, Epidemiology without biology: false paradigms, unfounded assumptions, and specious statistics in radiation science, Biol. Theory 11 (2016), 69–101.
[8] K. Shrader-Frechette, *Tainted*, Oxford University Press, Oxford 2014.
[9] https://en.wikipedia.org/wiki/Hormesis

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