Why is uncompetitive inhibition so rare?

A possible explanation, with implications for the design of drugs and pesticides

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Uncompetitive inhibition is much less common in nature than consideration of enzyme structure and mechanism might lead one to expect. A possible explanation may be that uncompetitive inhibition of an enzyme in a metabolic pathway can have enormously larger effects on the concentrations of metabolic intermediates than competitive inhibition, under circumstances where their effects on the kinetics of the isolated enzyme are very similar. The severely toxic effects that an uncompetitive inhibitor might be expected to have may have caused enzymes to have evolved in such a way that there has been selection against structures that might favour uncompetitive inhibition.

Enzyme kinetics   *Uncompetitive inhibition*   *Catastrophic response*   *Pesticide design*

1. INTRODUCTION

No one who has taught or written about enzyme kinetics and made a conscientious effort to provide examples of the kinds of behaviour regarded as important can fail to have been struck by the extreme paucity of examples of uncompetitive inhibition. It occurs, not particularly often, as a special case of product inhibition when the inhibiting product is isolated from the substrate by irreversible steps [1], but cases of uncompetitive inhibition by species that are not involved in the reaction are virtually unknown: the only example that I can find is the uncompetitive inhibition of alkaline phosphatase by L-phenylalanine [2]. Moreover, although mixed inhibition, in which both competitive and uncompetitive components are present, is quite common, the competitive component is nearly always dominant.

A conventional explanation might be that it is not particularly plausible in general to suppose that an inhibitor can only exert an effect on the enzyme-substrate complex and not on the free enzyme. While this may be valid as an explanation of why competitive inhibition is much more common than uncompetitive, it hardly explains the almost total absence of the latter. Moreover, it is not obvious why in cases where the inhibitor binds to both free enzyme and enzyme-substrate complex it normally binds much more tightly to the free enzyme. Although steric interference may account for some examples, one would expect there to be others in which ionic or conformational effects would cause the inhibitor to potentiate binding of substrate. Moreover, simple kinetic analysis indicates that an unreactive analogue of a substrate in a substituted-enzyme ('ping pong') mechanism should act as an uncompetitive inhibitor with respect to the other substrate, and that an unreactive analogue of the first product to be released in a compulsory-order ternary-complex mechanism should act in this way with respect to the first substrate. However, actual experimental examples of these kinds are hard to find; for illustrating uncompetitive inhibition one is usually forced to rely on extrapolating observable behaviour to

saturating (i.e. infinite) concentrations of one substrate, which does not, of course, represent a genuine example.

This raises the possibility that uncompetitive effects may not merely be mechanistically implausible but may be so detrimental to organisms that display them that there has been evolutionary selection against such inhibition by naturally occurring metabolites. It may therefore be worthwhile to point out that any metabolic pathway in which uncompetitive inhibition can occur can potentially respond catastrophically to the presence of the inhibitor, as I shall now discuss.

2. THEORY AND RESULTS

When considering isolated enzymes in vitro, it is almost universal practice to regard the rate of reaction as a dependent variable determined by the concentrations chosen by the experimenter. Atkinson [3] has cogently discussed why this is quite unrealistic for considering enzymes in vivo. As an alternative, he suggests that for many enzymes it is appropriate to regard the rate as the independent variable, with metabolite concentrations determined by it. The most realistic but more complicated approach is to compromise between this view and the conventional one, as I try to do below, but Atkinson's viewpoint provides a convenient and simple starting point for considering why competitive and uncompetitive inhibition should have entirely different consequences in vivo.

If a metabolite X is produced at a constant rate v and consumed according to Michaelis-Menten kinetics with maximum velocity V and Michaelis constant K_m, it is a simple matter to show that the steady-state concentration of X will be given by the following expression:

$$x_{ss} = \frac{K_m}{(V/v) - 1}$$

When an inhibitor is present, V and K_m must be replaced by 'apparent' values, V^{app} and K_m^{app}, respectively. For competitive inhibition, K_m^{app} is a linear function of the inhibitor concentration, but V^{app} is independent of the inhibitor; thus in this case x_{ss} is a linear function of the inhibitor concentration. If the inhibition is uncompetitive,

however, with inhibition constant K_{iu}, then

$$K_m^{app} = K_m/(1 + i/K_{iu})$$

$$V^{app} = V/(1 + i/K_{iu})$$

and $\bar{x}_{ss}$ is given by an expression that has no finite positive value unless i/K_{iu} is less than $(V/v - 1)$:

$$x_{ss} = \frac{K_m}{(V/v) - 1 - (i/K_{iu})}$$

Various authors [4–6] have commented that in vivo substrate concentrations are likely to be somewhat below the appropriate K_m values, and it has sometimes been suggested that one should expect them to be higher [7]. In any event they are likely to be of the same order of magnitude, and it is not unreasonable as a first approximation to put $v = 0.5V$, or $V/v = 2$, which would imply that no steady state exists if i exceeds the inhibition constant K_{iu}.

As this result is at first sight very surprising, it may be helpful to try to rationalize it in conceptual terms. Whenever a step in a metabolic process is inhibited, one of the first effects will be an increase in concentration of the substrate or substrates of the inhibited step. This will happen regardless of the type of inhibition, but the secondary effects will be highly dependent on the type of inhibition: if it is competitive, an increasing substrate concentration will tend to overcome it, and so the system is able to adjust with little difficulty; if it is uncompetitive, however, the presence of a term in *is* in the rate equation means that the substrate tends to potentiate the inhibition, and the more the substrate concentration rises the more the reaction approaches the limiting-rate condition in which uncompetitive inhibition is most effective.

As mentioned above, however, this constant-rate case is as much of an extreme as the more usual constant-concentration case. In a real metabolic system it is no more valid to take the concentrations as determined by the rates than to take the rates as determined by the concentrations; instead, it is now becoming recognized that control is shared between all elements of the system [8,9]. This complicates the analysis but it does not invalidate it, as may be seen from the curves plotted in fig.1. These show the concentration of the substrate of the fifth enzyme in a linear pathway as
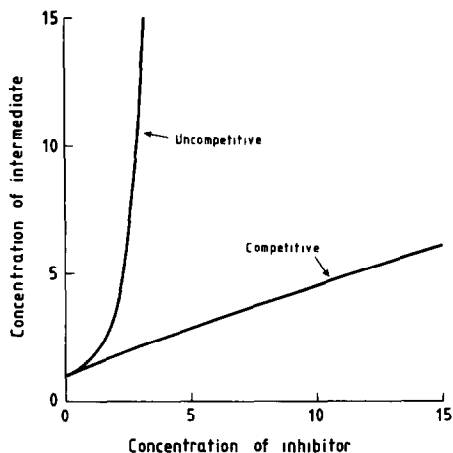
Fig.1. Different effects of competitive and uncompetitive inhibitors on the concentration of an intermediate in a metabolic pathway. The pathway consists of a linear sequence of enzyme-catalysed reactions from A to K, each with an equilibrium constant of 10 in favour of product, and proceeding at a rate equal to $(10s - p)/(1 + s + p)$, where s and p are the concentrations of the substrate and product of the reaction in question. In the case of the fifth reaction, the rate is $(10s - p)/(1 + s + p + i)$ for competitive inhibition by an external species at concentration i, or $(10s - p)/(1 + s + p + si)$ for uncompetitive inhibition. The concentrations of A and K were set at 1.035 and 0, respectively, values that give a value of 1.000 for the concentration of the substrate of the fifth enzyme in the absence of inhibitors. At these concentrations and in the absence of inhibition the flux control coefficients for the ten enzymes are, in the order in which they appear in the pathway, 0.424, 0.245, 0.141, 0.081, 0.046, 0.026, 0.014, 0.008, 0.004 and 0.001. The computer program used for simulating the pathway is described and reproduced in full elsewhere [13].

a function of the concentrations of external competitive and uncompetitive inhibitors with inhibition constants equal to the substrate concentration that exists in the absence of inhibition. Both curves have the same tangent at the axis, i.e. the same initial slope, but whereas the curve for the competitive inhibitor is almost straight, the one for the uncompetitive inhibitor is highly curved and rises very steeply at inhibitor concentrations not much larger than K_{iu}. For both types of inhibition the substrate concentration eventually levels out at the equilibrium value of 10000.

3. DISCUSSION

The degree to which the behaviour of an intermediate in a metabolic pathway resembles the theoretical case for a constant rate depends in part on how far its normal physiological concentration is removed from equilibrium with the reservoir of precursor. In the example illustrated in fig.1, the equilibrium value of the substrate concentration for the inhibited enzyme was 10000-times greater than its normal value. In a real pathway the corresponding values will vary widely, but in general we can expect that intermediates separated by several steps from the precursor pool the factor will be large. For example, in the presence of hexokinase, hexose-phosphate isomerase and phosphofructokinase but not aldolase the concentration of fructose 1,6-bisphosphate could in principle rise to about 3×10^6-times the glucose concentration, assuming an [ATP]/[ADP] ratio of about 5, if other effects did not intervene.

In addition one must consider the flux control coefficient $C^v_{E_i}$ [10,11] of the inhibited enzyme, i.e. the partial derivative of the logarithm of the pathway flux v with respect to the logarithm of the concentration E_i of the inhibited enzyme:

$$C^v_{E_i} = \frac{\partial \ln v}{\partial \ln E_i}$$

In the pathway illustrated in fig.1, enzyme 5 has a flux control coefficient of 0.046, but enzymes later in the pathway have lower values and resemble the simple case more closely, whereas enzymes near the beginning of the pathway have higher values and show comparatively small effects for either kind of inhibition. In every case, however, an uncompetitive inhibitor has a much larger effect than a competitive inhibitor on the concentration of the substrate of the inhibited enzyme.

The pathway illustrated is somewhat artificial (all enzymes obeying the same kinetics with the same equilibrium constant, etc.), but its characteristics were chosen to facilitate concise definition rather than to produce a particular result; qualitatively similar behaviour can easily be generated with more realistic pathways. It may be questioned, however, whether it is realistic that the flux-control coefficients for the later enzymes in the pathway are all very small. A general answer to

this will have to await a much larger body of experimental data than exists at present. However, it is of interest that a recent study of the metabolism of the aromatic amino acids [12] showed under a variety of conditions that control resided almost wholly in the first two steps of the pathway (including transport across the plasma membrane as the first step).

The implication that uncompetitive inhibition by naturally occurring metabolites is so detrimental to living organisms that it has been actively selected against is not, unfortunately, easy to test experimentally. Nonetheless, the theory outlined here has important practical implications for the design of toxic substances, e.g. for pest control. Naively, one might suppose that it would be best to try to find a compound that acted as a competitive inhibitor on a 'key' enzyme in a major metabolic pathway (more properly, an enzyme with a large flux control coefficient for the flux through its own reaction). Unfortunately, however, this strategy is likely to give a compound of negligible toxicity, because competitive inhibition within a pathway has little effect on either rates or concentrations, and because inhibition of the enzyme with the highest flux control coefficient has comparatively little effect on metabolite concentrations. Instead one should try to find an uncompetitive inhibitor of an enzyme with a very small flux control coefficient. Although this will certainly be much more difficult, it is likely to be far more rewarding, because any such inhibitor is much more likely to have a major toxicological or pharmacological effect.

The analysis is not greatly complicated by considering mixed inhibition instead of uncompetitive, because the competitive component in the inhibition has little practical consequence for this purpose. The essential point is that severe toxic effects of an inhibitor are likely to occur if the uncompetitive inhibition constant is low enough to be exceeded by the inhibitor concentration.

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