Biochemistry

 Volume 36, Number 36 September 9, 1997

New Concepts in Biochemistry

Enhanced Detoxication Due to Distributive Catalysis and Toxic Thresholds: A Kinetic Analysis†

Daniel L. Cook‡ and William M. Atkins*

Department of Biophysics and Physiology, University of Washington, and RainTown Biotech, P.O. Box 31263, Seattle, Washington 98103-1263, and Department of Medicinal Chemistry, Box 357610, University of Washington, Seattle, Washington 98195-7610

Received May 29, 1997; Revised Manuscript Received July 18, 1997

Predictive models that relate optimal enzyme function to experimentally measurable parameters have been based on net flux of substrate to product or flux of metabolites within a multienzyme pathway (*1*−*5*). These models define kinetic and thermodynamic features of individual enzymatic reactions, or metabolic pathways, that optimize rates of chemical conversion of a specific substrate to a single product or control of the flux. However, these theories require significant expansion to accommodate detoxication enzymes, which collectively metabolize an extraordinary range of environmental toxins and drugs. The realization that detoxication enzymes, such as cytochrome P-450s (P450s), glutathione S-transferases (GSTs), UDP-glucuronosyl transferases (UGTs), and flavin monoxygenases (FMOs), do not fit into the paradigms of substrate-specific enzymes has prompted useful, but limited, models of detoxication catalysis (*6*−*8*). In fact, Knowles et al. (*2*) have suggested that understanding the optimal performance of detoxication enzymes "is hopeless" as long as a specific substrate, and hence a biological niche, cannot be identified. The biological niche of detoxication enzymes is obscured further by recent appreciation for their ability to bioactivate environmental chemicals to more toxic metabolites (*9*−*13*). That is, detoxication enzymes are double-edged swords that can either catalyze the elimination of a toxin, or the formation of a more toxic metabolite.

The power of detoxication enzymes frequently has been attributed to their abilities to metabolize, individually as well as collectively, a broad range of structurally unrelated substrates. The remarkable substrate diversity is often cited as a distinguishing feature of these enzymes, compared to the substrate-specific enzymes involved in intermediary metabolism and homeostasis (*6*−*8*). Furthermore, it has been suggested that this substrate diversity is incompatible with fast turnover rates, and these enzymes are, therefore, characterized by slow V_{max} values, compared to substrate-specific enzymes.

However, an underappreciated feature of the detoxication enzymes is the multiplicity of products generated in many cases from a single [enzyme·substrate] complex. A single enzyme may produce several chemically distinct products from a single substrate (including regio-and stereo isomers; *14*−*16*), and this feature provides an additional distinction of detoxication enzymes. Apparently, this feature has been considered to be a natural consequence of their substrate diversity, with little regard for the biological utility of product diversity.

Thus, we are left to account for the evolution of detoxication enzymes that appear to be very poorly designed in contrast to highly specific enzymes operating in other metabolic pathways. Is there a selective advantage of detoxication enzymes that are generally slow, can metabolize multiple substrates, and may produce multiple products from a single substrate? Characterized this way, one can imagine that even a single detoxication enzyme could create a distributed catalysis network wherein a single substrate is distributed into multiple products, each with a lower concentration than that of the original substrate. Here, we present kinetic simulations to demonstrate how enhanced

† W.M.A. is supported by The National Institutes of Health (GM51210-01A1) and Merck Research Labs.

* Corresponding author. Phone: (206) 685-0379. Fax: (206) 685-3252. E-mail: winky@u.washington.edu.

‡ Department of Biophysics and Physiology and RainTown Biotech.

S0006-2960(97)01284-1 CCC: $14.00

detoxication may result from such systems of distributed catalysis of toxins having toxic thresholds and independent molecular mechanisms of toxicity. The advantage of distributive catalysis is clarified by comparing two situations.

If a species is exposed chronically to the same xenobiotics, selective pressure will optimize performance of the enzymes which provide the safest elimination pathway in much the same way as metabolic enzymes are selected for. If metabolites of the compound are more toxic than the parent compound, then these bioactivation pathways will be selected against, in favor of other pathways that provide higher net flux through the safe pathways via optimization of k_{cat}, K_M, or adjustment of internal equilibrium constants (K_{int}) for the particular substrate and a particular product bound to the enzyme (*1*−*5*). Also, the adaptive response may include genetic induction of the enzymes involved in the safe pathway of detoxication, as observed for many of the detoxication enzymes (*12*, *13*, *17*). Thus, with sustained exposure to an environmental toxin, selective pressure may optimize the metabolism of the compound in the same ways that substrate-specific enzymes are optimized.

However, the more realistic situation we must consider is that species face a nonconstant, time-dependent, array of xenobiotics. Are there characteristics of detoxication enzymes that can optimize their performance in detoxication of compounds to which a species has not been previously exposed? The substrate diversity described above is one such trait. However, upon exposure to a xenobiotic not encountered previously, a *naive* species cannot know if any single metabolite that it can produce is more or less toxic than the substrate or any other candidate product. There will always be some probability that fast conversion of the compound to any specific metabolite will simply speed up, or intensify, the toxic response. Thus, maximizing the flux of substrates to products is unlikely to be the best evolutionary strategy for all detoxication enzymes when there is a time-dependent xenobiotic profile. In this case, the detoxication process becomes a probabilistic game, in which the species must minimize the probability that the compound will cause toxicity. Aspects of this probabilistic game are considered here, as components in the optimal performance of detoxication enzymes, and it is demonstrated that multiple product formation represents a catalytic device for detoxication enzymes.

METHODS

Models were created and analyzed using Kinecyte (Raintown, Seattle, WA), a cell biological kinetic modeling program that provides a graphic user interface for "drawing" biochemical or metabolic systems as a series of linked enzymes and reactions and simulating the dynamic behavior of the system.

The purpose of the model (Figure 1, top) is to simulate a system of distributed catalysis wherein a single detoxication enzyme (E) reversibly binds a toxic substrate ($E + S \rightleftharpoons E{\cdot}S$), converts the S into four products ($P_1...P_4$), and reversibly dissociates from each. As our goal is to demonstrate certain qualitative principles of system function, we have made several simplifying assumptions that can be relaxed as discussed below. First, models have been limited to four toxic products, and binding rate constants are identical for all products and the initial toxin S. Second, we have set the

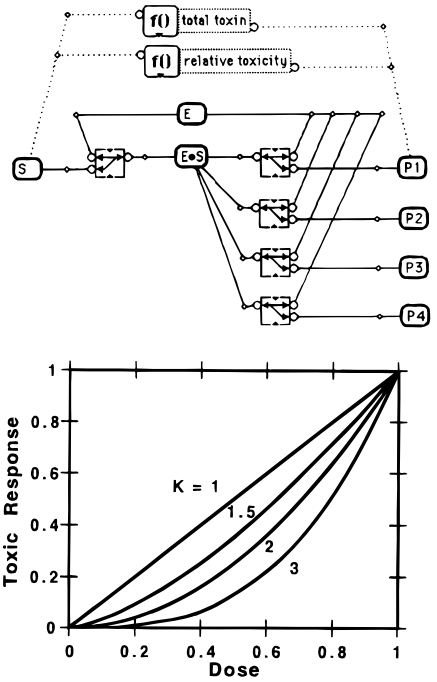

FIGURE 1: (Top) Kinetic model of a system of distributed catalysis. The model simulates the conversion of substrate (S) to each of four products ($P_1...P_4$) by a reversible enzymatic process. E and E·S, are free enzyme and enzyme−substrate complex, respectively. (The enzyme−product complexes, $EP_1...EP_4$, are included in the E·S species). The functions "total toxin" and "relative toxicity" are the sums $S + \sum P_i$ and $[(S/S_0)^K + \sum(P_i/S_0)^K]$, respectively. The solid lines represent pathways of flux, whereas the dotted lines represent passive functions that perform an operation but do not alter the kinetics of any process. (Bottom) Shapes of toxic dose−response curves for $K = 1$, 1.5, 2, and 3. The sublinear dose−response curves ($K > 1$) approximate toxic thresholds of increasing steepness. See Methods for details.

initial concentrations of $P_1...P_4$ to zero, and S and E to 1 and 0.1 μM respectively. All first-order reaction rate constants were set to 1 s^{-1}, and second-order rate constants were 1×10^6 M^{-1} s^{-1}, or to zero when individual metabolic pathways were eliminated. For example, in a model in which formation of a single product, P_1, was formed from S ($i = 1$), the rate constants for pathways yielding P_2, P_3, and P_4 were set to zero.

Also, we calculate the total relative toxicity (Rel Tox) to the organism of the five toxins (S, $P_1...P_4$). For each toxin, the nonlinear or threshold behavior of its toxicity dose−response curves is approximated as simple power functions $(S/S_0)^K$ or $(P_i/S_0)^K$ where K is a Hill coefficient for the toxic response curves for substrates or products normalized to an arbitrary initial substrate concentration, $S_0 = 1$ μM (Figure 1, bottom). Note that K is *not* a Hill coefficient for the E responsible for catalysis in the model. The total relative toxicity, then, becomes $(S/S_0)^K + \sum(P_i/S_0)^K$ where i varies from 1 to 4 depending on the number of products, assuming S and $P_1...P_4$ do not compete for the same, unspecified toxic receptor. This definition of relative toxicity is not dependent on any specific molecular events, but is applicable in any case where a measured toxic endpoint (% mortality, fraction protein molecules inhibited, etc.) is proportional to receptor occupancy and follows standard receptor theory (*20*, *21*).

RESULTS

The premise on which models are based is that there is an evolutionary pressure for some enzymes in the detoxi-

cation pool to retain features which minimize the probability that a previously unencountered compound will cause toxicity. Thus, features which minimize this probability will be maintained in the population. This must be distinguished from the evolutionary pressure that selects for the safest, most efficient, metabolic pathways for elimination of an environmental toxin to which a species has been exposed previously. Obviously, if such pathways can be improved or induced and if the exposure remains sufficiently long on the evolutionary time scale, these pathways will be selected for.

Because there are numerous examples of bioactivation of exogenous compounds to more toxic species, it cannot be assumed that the relative toxicity of any enzyme-generated product will be less than the initial substrate. It must be assumed, *a priori*, that a previously unencountered substrate and each of the products produced by a detoxication enzyme from this substrate are equally toxic. Furthermore, not all enzyme-catalyzed detoxication reactions are irreversible (*8*, *18*, *19*), and the scheme in Figure 1 may be interpreted as physiologically unrealistic for cytochrome P-450s, FMOs, and others. However, the issue of reversibility has been considered explicitly in other models of enzyme optimization (*1*−*4*), so the reverse pathways are included here. Importantly, the same conclusions derived from the present model are obtained whether the detoxication pathway is reversible or irreversible. Reversibility simply adds to the total number of species present at equilibrium or during steady state.

A key element underlying enhanced detoxication in our model is the nonlinear, threshold-like shape of the dose−response curves for environmental toxins and drugs. There is recent appreciation that toxin dose−response curves are often not linear over a wide concentration range, although prediction of potential risks of low dose exposures have been based on linear extrapolation from toxic levels to low levels (*20*, *21*). In many cases, this extrapolation overestimates the risk, suggesting that dose−response curves can be sigmoidal, or have toxic thresholds (*20*, *21*). Because data for low dose exposure are not widely available, we have examined the effect of increasing degrees of nonlinearity of the dose−response curve when a potential toxin is metabolized to multiple products (Figure 1, bottom). We have not considered K values <1 here, although supralinear dose−response curves are, in principle, possible. It is also important to distinguish between sigmoidal dose−response curves and thresholds. Formally, a threshold refers to the highest concentration tolerated with no toxic response. In contrast, a sigmoidal dose−response curve may afford a toxic response at all nonzero concentrations of toxin, but with increasing steepness of the dose−response curve. Here, we use the sigmoidal dose−response curve to approximate a toxic threshold. The same qualitative conclusions are expected if, instead, a threshold is used and the terms are used interchangeably here.

Another assumption utilized is that any substrate or product that is complexed to the detoxication enzyme will not contribute to the toxicity. During steady state, after a single dose or sustained exposure to an environmental toxin, substrates and products bound to detoxication enzymes are considered to be nontoxic because they are not available to bind to proteins, DNA, or other cellular nucleophiles.

One further simplifying assumption is that the probability that any product is toxic is independent of i. It may be argued that, as the number of products i increases, then the probability that at least one species, or their combination, is more highly toxic also increases. However, for reasons outlined in the Discussion, these probabilistic issues are unlikely to be important here.

Case 1: A Single Dose. The first case considered is that of exposure to a single bolus of toxic substrate, S. First, it is necessary to confirm the accuracy of the model calculations by monitoring the concentrations of substrate and products, and demonstrating that they behave as expected. For the sake of this demonstration, a test case with 3 products is shown in Figure 2, where the initial concentration of substrate is 1 μM. In panels A and B of Figure 2, the concentrations of the substrate and one of the products, P_1, are shown at time 0−100 s. As expected for a completely reversible system with three products, the concentration of substrate and each of the products is ~0.23 μM at equilibrium. More interestingly, the concentration of total toxins free in solution rapidly reaches equilibrium at 0.93 μM, slightly below the initial substrate concentration of 1.0 μM (panel C), due to sequestration of 0.07 μM products as E·S, which includes $E{\cdot}P_1$, $E{\cdot}P_2$, and $E{\cdot}P_3$ complexes. As expected, these kinetics of distribution are independent of the value of K which is used solely for estimating total relative toxicity.

In contrast, however, the relative toxicity resulting from the equilibrium distribution of metabolites decreases as K increases from 1 to 3 (panel D), and it is reduced further with additional increases in K. Even the mildest degree of nonlinearity ($K = 1.5$) reduces the relative toxicity to less than half of the toxicity observed for $K = 1$. This result is the focus of the subsequent calculations below, and it provides significant new insight into the biological niche of detoxication enzymes.

In order to determine the relationship between K, i, and toxicity, simulations analogous to those in Figure 2 were performed with variable K and i. The results demonstrate that if the toxic dose−response is linear ($K = 1$), then the relative toxicity does not change with increasing i, because the relative toxicity is simply proportional to the sum of the concentrations of the individual species that are free in solution at equilibrium. Notably, sequestration by the enzyme does provide a modest benefit, as shown in Figure 2D, but the extent of this benefit is independent of i. The potential utility of toxin sequestration by some detoxication enzymes has been suggested (*22*).

However, if the relative toxicity vs dose is even moderately nonlinear with $K = 1.5$ (see Figure 1B), or greater (i.e. threshold or sigmoidal response), then the total relative toxicity becomes a sensitive function of the number of products as summarized in Figure 2E, which shows the relative toxicity at equilibrium as K varies from 1 to 3.5 and when i varies from 1 to 4. For all K values, the time-dependent substrate and product concentration for each value of i (not shown) are the same as observed in panels A and B, respectively, of Figure 2, because the value of K does not affect the equilibrium values of the individual species, the rates at which equilibrium is obtained, or the concentration of total toxins (Figure 2C). This is expected, as the K value should not affect the degree of sequestration by the detoxication enzyme. However, when K is 1.5, as for a modestly nonlinear dose−response, a significant reduction in relative toxicity is observed as i progresses from 1 to 4 (Figure 2E). As K increases further, the advantage of generating multiple products also increases, until K ap-

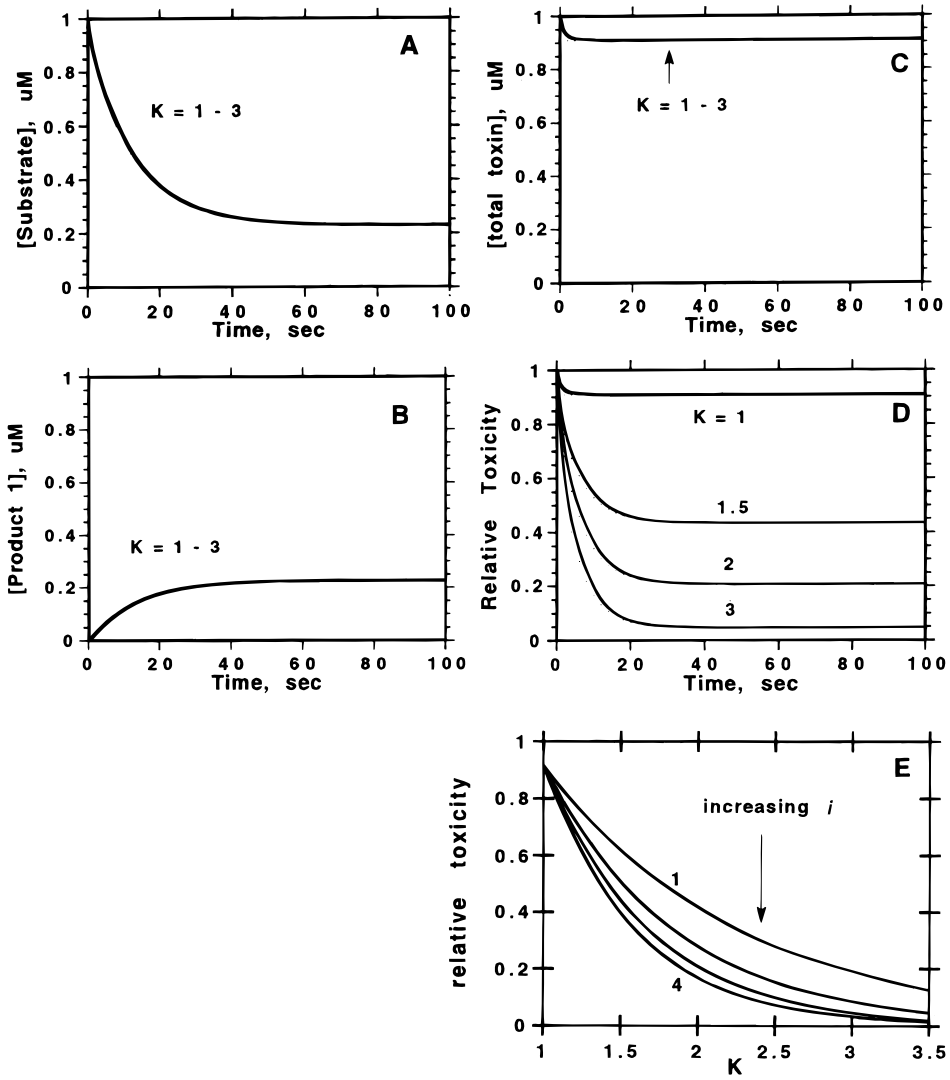

FIGURE 2: Kinetic simulation of concentrations of S (panel A), P_1 (panel B), total toxin (panel C), and relative toxicity (panel D) vs time after a single bolus of S, when K varies and $i = 3$. At $K > 1$, there is decrease in relative toxicity at equilibrium as K increases. Panel E shows the relative toxicity at equilibrium after a single bolus of S as a function of K, for $i = 1...4$. At $K = 1$ the toxicity is independent of i. For $K > 1$, the equilibrium toxicity decreases as i increases.

proaches 2.5. At this point, the equilibrium toxicities converge near zero for all values of i. Thus, for the case of a single dose of toxin, it is evident that there may be enhanced detoxication when distributed catalysis generates multiple products that have sublinear toxic dose−response curves.

Case 2. Sustained Exposure. In the second case, a constant influx of substrate is supplied (at 1 μM/s) starting at time zero. In order to allow the substrate and product concentrations to come to a steady-state, a first-order nonenzymatic decay process was added (half-time = 10 s) for S and each P. Because there is a net flux of metabolite through the system, the steady-state value of S is greater than each of the Ps. For comparison, a test simulation with variable K and $i = 3$ is shown in Figure 3, panels A−D, analogous to the simulation in Figure 2.

As with the case of a single bolus, the effect of variable i and K on toxicity was examined. At $K = 1$, the relative toxicity at steady state is independent of i. However, when a moderate toxic threshold is introduced, with $K = 1.5$, 2.0, or 2.5, the same qualitative result is observed as for the single dose. Results from kinetic simulations with $K = 3$ and variable i are shown in Figure 3E. Figure 3F also summarizes the results of simulations for variable K (1...3.5) and variable i (1...4), for the case of a steady state level of S. Again, an increase in the number of total products leads to changes in the steady state concentrations of substrate and products, but not their sum. Thus, the total concentration of toxins is unaffected by K. However, Figure 3 indicates that an increase in the number of products results in a decrease in the steady state relative toxicity when $K > 1$. In addition, examination of the individual kinetic simulations (Figure 3E) reveals a prominent lag time before the relative toxicity begins to increase. This lag time is accentuated by the presence of the toxic threshold, and is absent in the case of $K = 1$. Although the example used here has employed short times for convenience, the observed lag in the relative toxicity may be significant for minimizing the toxic response for slower processes. That is, for slower approach to steady state, the lag times will also be longer, and this may contribute to a decreases in the resulting toxicity. Most importantly, the effect of formation of multiple products is qualitatively the same for a single dose or a constant infusion of a potential toxin.

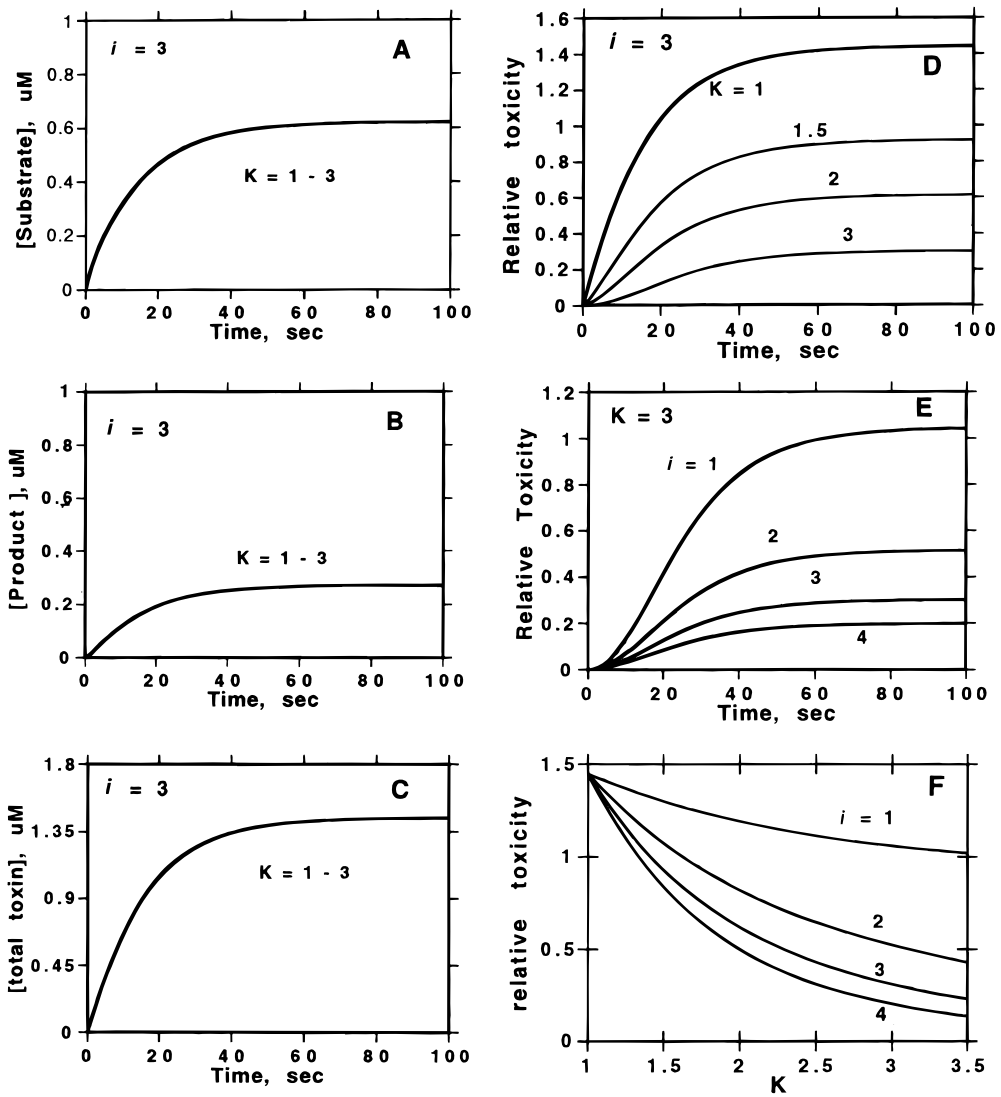

FIGURE 3: Simulation of S (panel A), P_1 (panel B), total toxin (panel C), and relative toxicity (panel D) vs time as the system approaches a steady state with a steady influx of substrate, when K varies and $i = 3$. The concentration of individual species in independent of K, as is their sum. However, the relative toxicity is a sensitive function of K, when $i = 3$. (Panel E) Kinetic simulation of relative toxicity vs time with a steady state level of S, when $K = 3$ and i varies. (Panel F) Summary of relative toxicity at steady state when K varies, for $i = 1-4$. At $K = 1$, the toxicity is independent of i. At $K > 1$, the relative toxicity decreases as i increases.

DISCUSSION

The models presented here demonstrate that the *a priori* probability that an organism will experience toxicity due to exposure to a new xenobiotic is minimized when the number of metabolites generated from the substrate increases, if toxic thresholds exist, and when the toxicity of any metabolite generated is equal to the toxicity of the starting xenobiotic. As a result of multiple product formation, potentially toxic metabolites are distributed so that the concentration of each is more likely to be below its own toxic threshold and, therefore, the contribution of each to total toxicity is reduced. Thus, the relative increase in the probability that the organism will avoid bioactivation of a less toxic compound by generating multiple products is greatest for the sharpest thresholds. That is, as the dose−response curves for the toxicities of a xenobiotic and its metabolites become increasingly sigmoidal, then the survival advantage associated with formation of multiple products increases. No advantage is obtained with multiple product formation for nonthreshold, hyperbolic, response curves as modeled by the case where $K = 1$. Importantly, the results demonstrate that a significant advantage may be obtained with formation of a second product: large values of i are not required. Indeed, if the enzymatic reaction is irreversible, as for most detoxication enzymes, then the advantage upon increasing i from 1 to 2 will be greater than observed here for the reversible reaction. These results, in turn, lead to the proposal that evolutionary pressure may select for detoxication enzymes which generate multiple products from a single substrate even if this means sacrificing catalytic efficiency (in k_{cat}/K_M terms). It is well documented that detoxication enzymes often generate multiple products from a single substrate (*14*−*16*). However, the biological advantage of generating multiple products has not previously been considered. The results presented here indicate that the criteria used to evaluate the optimal performance of detoxication enzymes should include the potential for bioactivation, distributive catalysis, and toxic thresholds in defining the biological niche.

The assumption that S and P_1...P_4 all have identical toxicity and are all formed at the same rate is unlikely to be true for any specific example of toxin metabolism. However, the results emphasized above remain qualitatively intact even if

these assumptions are relaxed. A higher concentration of one metabolite, due to a faster rate of formation, will lead to redistribution of the products at equilibrium or steady state, but the presence of additional enzymatic products will still provide a detoxication advantage, albeit with a decreased impact. Similarly, if one product is more toxic than the others, then larger values of *i* will be required to effectively lower the concentration of the more toxic species, or the less toxic species must be formed at a faster rate to effectively redistribute the toxic load. The essential point is that, for a previously unencountered toxin, a *naive* species can minimize the probability of bioactivation by forming multiple products, even if one product is more toxic than another or is formed at a faster rate.

Of course, it may be argued that the distributive catalysis described above is provided by several enzymes with overlapping substrate selectivity, rather than by a single enzyme. For example, identical results for the relative toxicity function are obtained if four different enzymes at one-fourth the concentration of E used here each produce a single product from S. Indeed, the toxicological advantage of multiple product formation would be the same, and this aspect of toxin metabolism is likely to be an important component of these enzyme systems. However, to the extent that evolution selects for the most efficient trait that affords an advantage, and the greatest efficiency is approached with the fewest enzymes, it is reasonable to speculate that a single enzyme affording multiple products would be selected for, as long as no substrate diversity was forfeited.

If product diversity is, in fact, a trait of detoxication enzymes that is selected for by a time-dependent xenobiotic profile (on the evolutionary time scale), then we are forced to consider how this could occur. What physical properties of an enzyme active site would afford product diversity? Obviously, substrate and protein dynamics that allow for multiple substrate binding orientations would accommodate formation of multiple metabolites. Indeed, it has been suggested that detoxication enzymes do not form specific complexes with substrates, but rather they invest binding energy into stabilization of high-energy, reactive, cofactors, such as the hydroperoxy flavin in FMOs, the glutathione thiolate in GSTs, or the perferryl-oxo heme in P450s (*6*−*8*). Presumably, in these cases, substrates sample multiple orientations at the active site and bump into the reactive cofactor. Indeed, rapid reorientation of substrate within the active sites of cytochrome P450s is suggested by intramolecular deuterium isotope effects (*14*, *23*−*25*). The models developed here indicate that substrate mobility within the active sites of detoxication enzymes is not simply a useless result of broad substrate selectivity. Rather, substrate dynamics may serve as a catalytic device that provides a biological function.

REFERENCES

1. Albery, W. J., and Knowles, J. R. (1976) *Biochemistry 15*, 5631−5640.
2. Burbaum, J. J., Raines, R. T., Albery, J., and Knowles, J. R. (1989) *Biochemistry 28*, 9293−9305.
3. Klipp, E., and Heinrich, R. (1994) *J. Theor. Biol. 171*, 309−323.
4. Pettersson, G. (1992) *Eur. J. Biochem. 206*, 289−295.
5. Ellington, A. D., and Benner, S. A. (1987) *J. Theor. Biol. 127*, 491−506.
6. Jakoby, W. B., and Ziegler, D. M. (1990) *J. Biol. Chem. 265*, 20715−20718.
7. Poulsen, L. L., and Ziegler, D. M. (1995) *Chem. Biol. Interact. 96*, 57−73.
8. Grimshaw, C. E. (1992) *Biochemistry 31*, 10140−10145.
9. Shimada, T., Gillam, E. M., Sandhu, P., Guo, Z., Tukey, R. H., and Guengerich, F. P. (1994) *Carcinogenesis 15*, 2523−2529.
10. Raucy, J. L. (1995) *Toxicology 105*, 217−224.
11. Nelson, S. D. (1982) *J. Med. Chem. 25*, 753−765.
12. Nebert, D. W., and Gonzalez, F. J. (1987) *Annu. Rev. Biochem. 56*, 945−993.
13. Whitlock, J. P., Jr. (1986) *Annu. Rev. Pharmacol. Toxicol. 26*, 333−369.
14. Korzekwa, K. R., Trager, W. F., and Gillette, J. R. (1989) *Biochemistry 28*, 9012−9018.
15. Gallagher, E. P., Kunze, K. K., Stapleton, P. L., and Eaton, D. (1996) *Toxicol. Appl. Pharmacol. 141*, 595−606.
16. Ebner, T., Meese, C. C., and Eichelbaum, M. (1995) *Mol. Pharmacol. 48*, 1076−1086.
17. Pickett, C. B., Williams, J. B., Lu, A. Y., and Cameron, R. G. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 5091−5095.
18. Meyer, J. D., Crease, D. J., and Ketterer, B. (1995) *Biochem. J. 306*, 565−569.
19. Armstrong, R. N. (1997) *Chem. Res. Toxicol. 10*, 2−18.
20. Hart, R. W., and Frame, L. T. (1996) *Belle Newsletter 5*, 1−16.
21. Lutz, W. K. (1991) *Adv. Exp. Med. Biol. 283*, 151−156.
22. Meyer, D J. (1993) *Xenobiotica 23*, 823−834.
23. Miwa, G., Walsh, J. S., and Lu, A. Y. H. (1984) *J. Biol. Chem. 259*, 3000−3004.
24. Jones, J. P., Korzekwa, K. R., Rettie. A. E., and Trager, W. F. (1986) *J. Am. Chem. Soc. 108*, 7074−7079.
25. Darbyshire, J. F., Gillette, J. R., Nagata, K., and Syiyama, K. (1994) *Biochemistry 33*, 2938−2944.

BI971284B