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Metabolic ménages à trois: what does it mean for drug design?

It is with considerable interest that I read the recent article in *Drug Discovery Today* by William Atkins [1] about the allosteric kinetics of cytochrome P450 enzymes. Although this topic has been of some academic interest for the past 16 years [2], it is only recently that those involved in drug discovery have become interested. Part of the interest was generated by the large amount of *in vitro* data that is being generated by pharmaceutical companies.

The strategy of HTS is dependent on whether a single substrate can be used to probe the affinity of potential drugs for P450 enzymes, or if several substrates are required. These are important measurements that hint at the possibility for drug interactions and possible phenotypic variations in a drug's half-life. The plethora of binding motifs (heterotropic, homotropic, Michaelis) described by Atkins means that these measurements are filled with potential problems. Sophisticated screening designs are required to overcome these problems. The real question is, is it worth the cost?

Another reason for the increase in interest is that drug discovery has undergone a concurrent increase in interest in *in silico* modeling of drug metabolizing enzymes, and the P450 enzymes in particular [3]. At present, multiple substrate binding is not considered by most of these models. To predict binding affinity for two potentially non-distinct and overlapping substrate binding regions is problematic. Again the question is, is it worth the cost?

In essence we are asking the age-old question, does size matter? Atkins outlined two conceptual frameworks within which allosteric effects can be studied. The first is a kinetic approach, which does not readily distinguish between remote site activation/inhibition, or two substrates binding in the same or closely related active sites [4]. The second is a molecular approach, which has a goal distinguishing these two binding possibilities [5]. If two substrates can bind in the same active site, and that active site has a limited amount of ability to reorganize, size matters and we should, given enough information, be able to map these active sites and develop predictive models for affinity. If however, the enzymes can undergo large changes in conformation to accommodate multiple substrates, building affinity models for two-substrate binding might prove difficult.

Although most of the evidence indicates that two-substrates bind in a single shared active site, the possibility of a remote allosteric effect remains to further complicate the picture. The organizational complexities of such a foursome are mind-boggling. Thus, I would strongly encourage efforts using the second approach to establish a database that will enable drug designers to determine if it is worth the cost to model two-substrate binding.

The next important question that was raised by the article by Atkins is how the *in vitro* effects correlate with *in vivo* effects. An important point was made in that even if allosteric effects are not important *in vivo* '… it is a pervasive behavior *in vitro*' and an understanding of the behavior is important for *in vitro–in vivo* correlations. In fact, the limited number of observations of *in vivo* allosterism with P450 enzymes is, in most cases, a matter of concentration. To easily observe non-Michaelis kinetics the concentration must be varied over a large range. Most *in vivo* studies do not cover the higher concentrations required for two-substrate binding. As our tools become more sophisticated, I am certain that we will find more cases *in vivo*. At present, the tools are just being developed and put into place to establish the foundational knowledge. Soon we might not be so married to Michaelis kinetics and we might be practicing a more interesting form of kinetics.

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Jeffrey P. Jones
Department of Chemistry
Washington State University
Pullman, WA 99164-4630, USA
e-mail: joswigjones@earthlink.net

PII: S1359-6446(04)03109-5