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In this paper hysteretic enzymes are defined as those for which the kinetic behavior responds slowly to a rapid change in ligand concentration. It is noted that a number of allosteric enzymes show this property and it is suggested that such behavior is important in metabolic regulation. [The *SCI*® indicates that this paper has been cited in more than 430 publications.]

Defining Hysteretic Enzymes

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The decade of the 1960s was an exciting time for those of those of us interested in enzyme kinetics. In the early part of that decade, the concept of allosteric enzymes had been developed and it was becoming clear that much of metabolic regulation was a consequence of unusual kinetic properties of these enzymes. J. Monod et al.[1] and D.E. Koshland et al.[2] had developed kinetic theories to explain these regulatory effects based on the idea of conformational changes occurring as a consequence of ligands binding to sites other than the active site of the allosteric enzyme. As one of the early examples, we had found that bovine liver glutamate dehydrogenase was inhibited by high levels of NADH as well as by GTP and GDP but activated by ADP, nucleotides clearly binding to nonsubstrate sites.[3] In addition, the enzyme was a self-associating system and different molecular weight forms bound nucleotides with different affinities—another mechanism for regulating enzymatic activity. As we were examining the dissociation of the enzyme induced by GTP in the presence of NADH, we noted that it was time dependent.[4] I was acclimated, by that time, to the role of conformational changes affecting the kinetic behavior, but allosteric theories were developed based on binding, not kinetic, properties with the implicit assumption, when applied to enzymes, that conformational changes were rapid relative to activity measurements. It was time to consider the possibility that the rate of the conformational change from one kinetic form to another was not instantaneous but could take time—thus the hysteretic concept.

Why "hysteresis"? That term seemed to fit because the Greek definition means to be behind or to lag and that was exactly what was happening in the kinetic response. My mother, schooled in classical Greek, gave her approval. Indeed, I think that the term has become ingrained enough in the biochemical literature that many papers no longer include a reference to the original work. The fact that the same term is used in physics to describe magnetic induction properties with changing magnetic field strengths clearly hasn't been a deterrent to its use. A number of people, however, suggested that these enzymes should really be called hysterical (a rather different Greek root, of course).

The original paper discussed a number of reasons for time-dependent behavior—enzyme isomerization, displacement of tightly bound ligand, or ligand-induced changes in molecular weight. An equation describing the time course of product formation was derived for these conditions and later found to describe the case for slow binding processes (e.g., transition state analogs) as well. But it was clear that if the activity of an enzyme were time dependent, how does one determine the initial velocity of the reaction? Solving this puzzle led to the development of a computer program that would allow one to type in complex mechanisms and then solve, by numerical integration, for the full time course of the reaction. David Bates, a graduate student, first developed such a program for a PDP-12/40. This program was later marvelously developed by Bruce A. Barshop for the VAX.[5] Barshop's program, and its modified versions, has been found to be extraordinarily useful as another tool in the investigation of time-dependent processes. Over the years we have provided these programs to all interested investigators, and I am now pleased to report that they are available via Internet (on WUARCHIVE) for both small and mainframe computers.

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2. **Koshland D E, Nemethy G & Filmer D.** Comparison of experimental binding data and theoretical models in proteins containing subunits. *Biochemistry—USA* 5:365-85, 1966. (Cited 1,220 times.)
3. **Frieden C.** Glutamate dehydrogenase. V. The relation of enzyme structure to catalytic function. *J. Biol. Chem.* 238:3286-99, 1963. (Cited 255 times.)
4. **Huang C Y & Frieden C.** Rates of GDP-induced and GTP-induced depolymerization and isomerization of the bovine liver glutamate dehydrogenase-coenzyme complex: a possible controlling factor in metabolic regulation. *Proc. Nat. Acad. Sci. USA* 64:338-44, 1969.
5. **Barshop B A, Wrenn R F & Frieden C.** Analysis of numerical methods for computer simulation of kinetic processes: development of KINSIM—a flexible, portable system. *Anal. Biochem.* 130:134-45, 1983.

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