A UNIVERSAL BIOASSAY FOR IDENTIFYING ENZYME INHIBITORS

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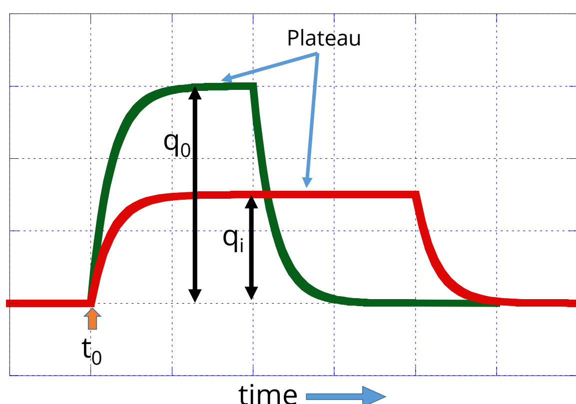
Dr. Freire is a world recognized expert in biological thermodynamics. He performs research in the thermodynamics of protein stability, protein-protein interactions, and protein-ligand binding. Dr. Freire has pioneered the development of drug design and optimization strategies using thermodynamic techniques. Dr. Freire is the author or co-author of over 260 publications and several patents. Dr. Freire is an Honor Member of the Spanish Society of Biochemistry and Molecular Biology, and a member of the Academy of Sciences of Latin America.

Most targets for drug development are enzymes and the search for small molecule inhibitors is a continuous and major endeavor in the pharmaceutical industry. Developing assays to measure enzyme activity and their inhibition by small molecules is often cumbersome, involving significant development time and investment. Often, developing an enzymatic assay requires the design of fluorescent substrates and sometimes the use of coupled reactions.

There is, however, a universal test for enzymatic reactions. It is well known that enzymatic reactions release energy in the form of heat and that this heat can be measured calorimetrically. The calorimetric test is universal and does not require an optically clear sample. It does not require especially designed substrates and utilizes the same amounts of enzyme as conventional assays.



In brief, if substrate is placed into the calorimeter reaction cell and at time zero a small amount of enzyme is added, the enzymatic reaction will begin and a curve like the green curve in the figure is observed. The calorimeter measures the rate of heat production as a function of time. Since the rate of heat production is proportional to the enzyme velocity or rate of substrate depletion (or product formation), at high substrate concentrations the plateau corresponds to V_{max}. If desired, the experiment can be performed at different substrate concentrations in order to determine K_m. Once the substrate concentration diminishes the signal also decreases until it reaches zero when the substrate is depleted. Under the same conditions but in the presence of an inhibitor the red curve is obtained. Note that the amplitude of the plateau (q_i) is smaller than the one observed in the absence of inhibitor (q_0). However, the area under both curves is the same since it is proportional to the total amount of substrate depleted. The degree of inhibition is simply ($1 – q_i/q_0$) thus providing a very easy way of evaluating the inhibitory potency of small molecules. Of course, more sophisticated analysis can be performed in order to determine the K_i. Calorimetric screening of small molecules at identical concentrations provides a fast and easy way of ranking a library of compounds in terms of their inhibitory potency.

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