A New Mathematical Model of Slow-Binding Enzyme Inhibition

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The treatment of pain and inflammation by the inhibition of prostaglandin formation has been successfully accomplished with many currently marketed NSAIDs (nonsteroidal anti-inflammatory drugs). The NSAIDs are thought to elicit their action by the inhibition of prostaglandin H synthase. However, severe GI irritation is also observed with the administration of these compounds, limiting their usage. This irritation has been associated with the inhibition of prostaglandin formation in the GI tract. The recent discovery of an inducible form of prostaglandin H synthase (PGHS2), induced at the sites of inflammation, has renewed interest in discovering new NSAIDs which will be better tolerated. The working hypothesis is that selective inhibition of the inducible enzyme will block the inflammation and the pain associated with the inflammation without serious GI side effects. To this end a great deal of effort is being spent to discover PGHS2 selective compounds.

It has been found that prostaglandin H synthase may be inhibited in a time-dependent manner and associated with a slow-binding mechanism involving a slow reversible isomerization of the initial enzyme-inhibitor. Although the inhibition has been reported to be reversible, the rate of the reverse isomerization has not been addressed. In addition, the kinetic and structural features which define the selectivity have yet to be determined. In this paper we present a method to determine the rate constants associated with inhibition of PGHS1 (the non-inducible form of PGHS) and PGHS2 which minimize the impact of the substrate-dependent auto-inactivation upon the slow-binding inhibition kinetics. The results indicate which kinetic events contribute to the overall affinity and selectivity of the inhibitors.

The mathematical model is subject to two phases, a preincubation phase in which inhibitor is incubated with enzyme in the absence of substrate, and a reaction phase which begins when the substrate is added to the inhibitor enzyme mix. For the preincubation phase, the model assumes a rapid reversible formation of an EI complex followed by a slow reversible formation of an EI* complex, where the EI and EI* complexes are in slow equilibrium. An equation for the final concentration of free enzyme and enzyme-inhibitor complex at the end of T seconds of preincubation is then developed. For the reaction phase, the model assumes the rate of inactivation from the enzyme-substrate complex and the rate of reverse isomerization from the EI* complex are slow relative to the other rates in the model and can be ignored in the initial dynamics of the reaction. Defining control as the

experiment performed with no inhibitor, and using the results from the preincubation phase, an equation for the maximum initial velocity after T seconds of preincubation as a percent of control is developed and found to be of the following form:

$$f(T) = 100Q\left(1 - \frac{k_{app}}{k_{app} + k_{-2}}(1 - e^{-(k_{app}+k_{-2})T})\right)$$

$$\text{where } Q = \frac{1 + \frac{K_m}{S}}{1 + \frac{K_m}{S}(1 + \frac{I}{K_I})}, \quad k_{app} = \frac{k_2}{1 + \frac{K_I}{I}},$$

where k_2 and k_{-2} are the forward and reverse isomerization rates respectively, and K_I and K_m are defined in the usual way. Nonlinear regression was used to fit collected data to f(T), estimating the three parameters k_2, k_{-2}, and K_I, using the known K_m/S and several inhibitor concentrations I. The overall inhibition was defined as $K_I^* = k_2/k_{-2}$, and the percentage of enzyme in EI* at saturation was determined to be $100*k_2/(k_2+k_{-2})$. Comparison of the various inhibitors with the two enzymes PGHS1 and PGHS2 show that all three kinetic parameters (k_2, k_{-2}, K_I) must be examined in order to understand which factors contribute to the overall affinities.

In summary, this paper presents a mathematical model to determine the kinetic constants associated with slow-binding inhibition of PGHS by various NSAIDs. This approach allows for the first time the determination of the reverse isomerization rates and the kinetic events which most influence the selectivity of inhibition of the two enzymes. The challenge now will be to use this information to design more effective and selective PGHS inhibitors and to determine the structural features which promote the slow-binding inhibition.