Biochemical and Biophysical Research Communications 445 (2014) 556–560

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Basic models for differential inhibition of enzymes

Mario Cappiello, Roberta Moschini, Francesco Balestri, Umberto Mura, Antonella Del-Corso *

Department of Biology, University of Pisa, Via S. Zeno, 51, Pisa 56127, Italy

ARTICLE INFO

Article history:
Received 28 January 2014
Available online 14 February 2014

Keywords:
Enzyme inhibition
Differential inhibition
Competing substrates
Aldose reductase

ABSTRACT

The possible preferential action exerted by an inhibitor on the transformation of one of two agonist substrates catalyzed by the same enzyme has recently been reported in studies on aldose reductase inhibition. This event was defined as "intra-site differential inhibition" and the molecules able to exert this action as "differential inhibitors". This work presents some basic kinetic models describing differential inhibition. Using a simple analytic approach, the results show that differential inhibition can occur through either competitive or mixed type inhibition in which the inhibitor prevalently targets the free enzyme. The results may help in selecting molecules whose differential inhibitory action could be advantageous in controlling the activity of enzymes acting on more than one substrate.

1. Introduction

The susceptibility to allosteric inhibition enables enzymes to respond to cell conditions in order to meet metabolic needs. Furthermore enzyme inhibition is one of the most common strategies used to control specific enzymes or metabolic pathways. Different models of enzyme inhibition, used to explain experimental inhibition data, focus on the relative ability of the inhibitor to interact with the free enzyme and/or with the enzyme-substrate complex. This approach leads to a pragmatic definition of the active and inhibition sites, and the related influence between them is considered as the rationale underlying the inhibitor's behavior.

A significant aspect of enzyme inhibition studies concerns the ability of several enzymes to act on different competitive alternative substrates. The first issue is to discriminate between the real ability of the enzyme to act on different substrates and the presence in the enzyme preparations of different enzyme molecules (i.e. isoenzymatic forms or modified forms). To solve this problem a methodological approach was proposed to analyze systems in which two substrates were simultaneously present, which provides insight on whether the transformations could be ascribed to one or to two different catalytic sites [1]. The analysis of a system consisting of one enzyme with two competing substrates using the steady-state kinetic approach leads to a kinetic equation which reveals an apparent mutual inhibitory effect of the two substrates undergoing transformation [2–5]. This analysis thus evaluates the real catalytic effectiveness of an enzyme on substrates simultaneously present in the biological systems. The effect of substrates competing for the same enzyme on metabolic pathways has been recently considered [6]. In addition the use of competing substrates has been proposed to determine the steady state kinetic parameters for the agonist substrate [7]. Similarly, other authors have used alternative substrates to probe multi-substrate enzyme mechanisms [3,8].

We recently proposed a new strategy regarding the inhibition of enzymes that are able to act on different substrates, in which the inhibitor selectively intervenes on the enzyme activity depending on the substrate the enzyme is working on. Such an intra-site specific inhibitory action was termed as "differential inhibition" and the effectors able to exert this action as "differential inhibitors" (DIs) [9]. The usefulness of this approach was highlighted in studies on aldose reductase (AR), an enzyme targeted for decades with specific and highly powerful inhibitors (ARIs) in order to find drugs for the prevention of diabetes complications (for a review see [10]). The ability of the enzyme to reduce both hydrophilic substrates, such as aldoses (thus giving rise to cell damage) and hydrophobic aldehydes, such as HNE (thus removing a cytotoxic product of lipid peroxidation), raised doubt as to the effective gain in inhibiting the enzyme. This doubt is supported by a general failure in developing effective drugs from ARIs. Indeed, we also proved the potential of AR to undergo differential inhibition [9], and a new generation of enzyme inhibitors, aldose reductase differential inhibitors (ARDIs), is under investigation.

The first step in revealing DIs for enzymes with respect to two different substrates involves evaluating the inhibitory effectiveness

* Corresponding author. Address: Department of Biology, Biochemistry Unit, via S. Zeno 51, Pisa 56127, Italy. Fax: +39 050 2211460.
E-mail address: adelcorso@biologia.unipi.it (A. Del-Corso).

http://dx.doi.org/10.1016/j.bbrc.2014.02.030
0006-291X/

of the inhibitor in terms of the two different substrates assayed separately. However the ability of the inhibitor to act as a DI must be more realistically verified by testing the inhibitory action when both substrates are present in the assay.

This paper presents the simple kinetic analysis of a system in which the catalytic activity of an enzyme on two agonist substrates is differently targeted by a DI.

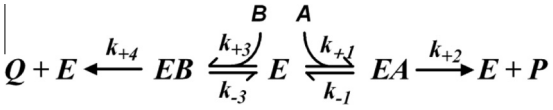
2. Methods

The present analysis assumes that classical Michaelis–Menten kinetics is obeyed, that agonist substrates are mutually exclusive and that the inhibitor combines reversibly with the enzyme and or with the enzyme substrate complexes. Rate equations at zero time, derived for different models of differential inhibitions, are graphically represented essentially by double reciprocal plots [11].

3. Kinetic models and discussion

3.1. Two agonist substrates for the same enzyme

The following scheme describes a reaction model in which two different substrates (*A* and *B*) are recognized and transformed into the corresponding products (*P* and *Q*, respectively) by the same enzyme (*E*) complying with a simple kinetic model:



(Scheme 1)

The steady state analysis of the enzymatic reactions [5] leads to the final kinetic equations (Eqs. (1) and (2)) for the transformation of the two substrates.

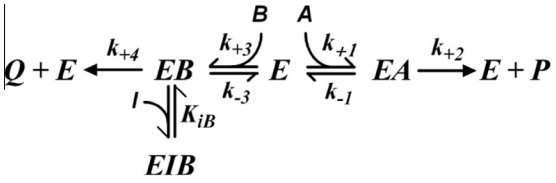
$$-\frac{d[A]}{dt} = v_p = \frac{V_A[A]}{K_A\left(1+\frac{[B]}{K_B}\right)+[A]} \tag{1}$$

$$-\frac{d[B]}{dt} = v_q = \frac{V_B[B]}{K_B(1+\frac{[A]}{K_A})+[B]} \tag{2}$$

In these equations $V_A = k_{+2}E_T$, $K_A = \frac{k_{-1}+k_{+2}}{k_{+1}}$, $V_B = k_{+4}E_T$ and $K_B = \frac{k_{-3}+k_{+4}}{k_{+3}}$. The terms V_A, V_B and K_A, K_B represent the maximum reaction rates and the Michaelis–Menten constants for the transformation of *A* and *B*, respectively, measured in the presence of the total enzyme concentration (E_T) in the absence of the agonist substrate. These equations highlight that when the two agonist substrates are simultaneously present, they reciprocally act as competitive inhibitors. In this model a differential inhibitor is defined as a molecule with the ability to specifically act only on the transformation of one of the two substrates. Kinetic models for ideal differential inhibitors were devised.

3.2. Uncompetitive differential inhibition

The simplest model describing the targeting of an inhibitor exclusively on one of the two competing substrates is reported in the following scheme, in which the action of the inhibitor (*I*) is limited to the binding to the complex *EB*, thus depicting for substrate *B* a model of an uncompetitive type of inhibition:



(Scheme 2)

This model admits that the structural differences between the two substrates will determine, once bound on the enzyme, the exposure of a different interacting surface for the inhibitor. Thus, only the *EB* complex is targeted by the inhibitor. The general kinetic equations for *A* and *B* transformation are:

$$v_p = \frac{d[P]}{dt} = k_{+2}[EA]$$

$$v_q = \frac{d[Q]}{dt} = k_{+4}[EB]$$

By applying the steady state condition for *EA* and *EB* and considering the complex *EIB* at the equilibrium, the following equations can be written:

$$k_{+1}[E][A] = (k_{-1}+k_{+2})[EA]$$

$$k_{+3}[E][B] = (k_{-3}+k_{+4})[EB]$$

$$K_{iB} = \frac{[EB][I]}{[EIB]}$$

Taking into account the mass balance for the enzyme:

$$E_T = [E]+[EA]+[EB]+[EIB]$$

a kinetic equation can be formulated (see Supplementary Material, Appendix I) for the transformation of both *A* and *B* (Eqs. (3) and (4), respectively)

$$v_p = \frac{k_{+2}E_T[A]}{K_A\left[1+\frac{[B]}{K_B}\left(1+\frac{[I]}{K_{iB}}\right)\right]+[A]} \tag{3}$$

$$v_q = \frac{k_{+4}E_T[B]/\left(1+\frac{[I]}{K_{iB}}\right)}{K_B\left(1+\frac{[A]}{K_A}\right)/\left(1+\frac{[I]}{K_{iB}}\right)+[B]} \tag{4}$$

As can be seen from Eq. (4), the transformation of substrate *B* in the presence of the agonist substrate *A* and the differential inhibitor *I* is affected both in terms of V_{max} as a result of the inhibitor action and in terms of apparent K_M as a result of the combined action of both the inhibitor and the agonist substrate (Fig. 1). In this case, changes in K_B^{app} values are linked to the relative values of the inhibitory impact factor for both the inhibitor $\left(1+\frac{[I]}{K_{iB}}\right)$ and the agonist substrate $\left(1+\frac{[A]}{K_A}\right)$. As also it emerges from Eq. (3), the inhibition of the transformation of *B* negatively affects the transformation of substrate *A*, although the latter was not directly targeted by the inhibitor. In fact the generation of the ternary complex *EIB*, by reducing the availability of the enzyme, leads to a general decrease in v_p. More specifically, at any finite value of both [*B*] and [*I*], K_A^{app} increases with the increase of both [*I*] and [*B*], while V_{max} remains constant (Fig. 2).