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Quantitative control analysis of branched-chain 2-oxo acid dehydrogenase complex activity by feedback inhibition

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The potential for branched-chain 2-oxo acid dehydrogenase complex (BCOADC) activity to be controlled by feedback inhibition was investigated by calculating the Elasticity Coefficients for several feedback inhibitors. We suggest that feedback inhibition is a quantitatively important regulatory mechanism by which branched-chain 2-oxo acid dehydrogenase activity is regulated. The potential for control of enzyme activity is greater for NADH than for the acyl-CoA products, and suggests that factors that alter the redox potential may physiologically regulate BCOADC activity through a feedback inhibitory mechanism *in vivo*. Local pH may also be an important regulatory control factor.

INTRODUCTION

Experiments were undertaken to quantify the control of branched-chain 2-oxo acid dehydrogenase complex (BCOADC) activity by feedback inhibition. The relative potential for a modulator to control the activity of an isolated enzyme can be determined by calculating its Elasticity Coefficient. The Elasticity Coefficient is defined as the fractional change in activity of an isolated enzyme divided by the fractional change in the concentration of an effector (substrate, product, inhibitor etc.), and is independent of the concentration of the enzyme catalysing the reaction (Westerhoff *et al.*, 1984). Only some of the kinetic constants for modulators of BCOADC have been measured in the past, and there is significant disagreement in their values. Therefore we thought it necessary to re-determine these kinetic constants from the same animal and tissue source, under physiological conditions, in order to make consistent quantitative predictions about their potential to inhibit BCOADC activity. In addition to the mitochondrial metabolites affecting BCOADC activity, we investigated the effect of pH on BCOADC activity, and characterized the effect of pH on the kinetic constants of the substrates and two inhibitors. This allowed us to define the effect of pH on the Elasticity Coefficient of the acyl-CoAs and NADH as feedback inhibitors of BCOADC activity.

With the use of purified BCOADC, it is shown that feedback inhibition is a quantitatively important mechanism by which branched-chain amino acid degradation can be regulated. Furthermore, on the basis of modulator Elasticity Coefficient calculations, the quantitative potential for feedback inhibition by NADH is greater than for the acyl-CoA products of the reaction. It is also suggested that the local pH of the mitochondrial inner membrane may be a quantitatively important regulatory mechanism.

METHODS

All enzyme assays were performed at 37 °C, and were essentially the same as described by Odessey (1980). All reactions were initiated by addition of 3-methyl-2-oxobutyrate (1 mM), unless otherwise indicated. Unless otherwise indicated, the assay buffer consisted of: NAD^+, 1 mM; thiamin pyrophosphate, 0.20 mM; CoA, 0.1 mM; dihydrolipoamide dehydrogenase, 0.061 unit/ml; rotenone, 0.5 μg/ml. To assay the modulators of the BCOADC, the assay buffer (1.0 ml total volume) was prepared with several concentrations of one cofactor, with fixed concentrations of competitive inhibitor being added just before the assay was to be performed. After the addition of the competitive inhibitor, 0.025 unit of purified BCOADC was added with mixing, and then 20 μl of 3-methyl-2-oxobutyrate (50 mM) was added with mixing. The production of NADH was monitored fluorimetrically for approx. 1 min to determine the initial rate of the reaction.

All kinetic constants were fitted to the non-linear Michaelis–Menten equation for competitive inhibitors as derived by Cleland (1970), and implemented on either an IBM 3081 mainframe using the MVS/XA operating system with the SAS non-linear-regression procedure, or an PCXT microcomputer using the Pennzyme non-linear-kinetics program generously supplied by Dr. David Garfinkel (Kohn *et al.*, 1979*b*). Competitive inhibition was assumed if there was no statistically significant difference in the $V_{max.}$ of individual curves at a single inhibitor concentration.

BCOADC was prepared from rat kidney mitochondria as described by Odessey (1980), with slight modifications (B. Boyer & R. Odessey, unpublished work).

The Elasticity Coefficients were calculated by entering the

Table 1. Oxo acid and cofactor K_m values for BCOADC at pH 6.8, 7.3 and 8.0

Kinetic constants were calculated as described in the Methods section. The substrate concentrations were varied from 5 μM to 200 μM, with the cofactors being held constant at the following concentrations: NAD^+, 1 mM; thiamin pyrophosphate, 0.2 mM; CoA, 1 mM; dihydrolipoamide dehydrogenase, 0.061 unit/ml. For further experimental details see the text.

Substrate	K_m (μM)		
	pH 6.8	pH 7.3	pH 8.0
3-Methyl-2-oxobutyrate	5.7±0.7	18.3±2.1	25.8±2.4
4-Methyl-2-oxopentanoate	9.0±1.1	16.7±1.4	17.2±1.4
3-Methyl-2-oxopentanoate	8.2±1.6	10.5±1.5	12.8±1.8
CoA	7.8±1.1	10.5±2.0	13.1±1.9
NAD^+	255.3±57.0	49.3±6.5	35.1±7.2

Abbreviations used: BCOADC, branched-chain 2-oxo acid dehydrogenase complex; PDC, pyruvate dehydrogenase complex.

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Table 2. Inhibitor K_i values and K_i/K_m ratios for BCOADC at pH 7.3

When the inhibitor was an acyl-CoA the CoA concentration was varied from 8 μM to 200 μM and the acyl-CoA concentration was varied from 5 μM to 30 μM. When the inhibitor was NADH the NAD^+ concentration was varied from 80 μM to 1.0 mM and the NADH concentration was varied from 2.5 μM to 10 μM. All other cofactors were at the concentrations given in Table 1, and 3-methyl-2-oxobutyrate (1 mM) was the oxo acid substrate. K_i/K_m ratios were calculated as described in the Methods section.

Inhibitor	K_i (μM)	K_i/K_m ratio
3-Methylbutyryl-CoA	7.4±1.0	1.42±0.3
Isobutyryl-CoA	17.1±7.5	1.4±0.5
β-Methylcrotonyl-CoA	5.2±0.9	0.36±0.05
Tiglyl-CoA	5.3±1.1	0.89±0.13
Acetoacetyl-CoA	11.1±2.4	1.1±0.2
Acetyl-CoA	14.6±4.3	1.5±0.3
Propionyl-CoA	11.9±3.2	0.95±0.2
Palmitoyl-CoA	0.2±0.03	0.04±0.004
Octanoyl-CoA	8.4±0.9	1.3±0.1
NADH	10.2±1.5	0.22±0.03

Table 3. 3-Methylbutyryl-CoA and NADH K_i/K_m ratios for BCOADC at pH 6.8, 7.3 and 8.0

Kinetic constants were calculated as described in the Methods section.

	K_i/K_m ratio		
Inhibitor	pH 6.8	pH 7.3	pH 8.0
3-Methylbutyryl-CoA	0.22±0.02	1.42±0.30	0.76±0.16
NADH	0.020±0.004	0.22±0.03	0.17±0.03

values of the kinetic constants and ratios (Tables 1–4) into the formula derived in the Appendix (eqn. 1). In all cases the oxo acid concentration was assumed to be approximately equal to its K_m. The Elasticity Coefficient for a competitive inhibitor on a Michaelis–Menten enzyme [e.g. BCOADC and pyruvate dehydrogenase complex (PDC)] can range from 0 to −1.0. A value of −1.0 means that a 1% increase in inhibitor concentration will cause a 1% decrease in activity. Conversely, a value of −0.01 predicts that a 100% increase in inhibitor concentration would be needed to cause a 1% decrease in enzyme activity. The Elasticity Coefficient is defined for very small incremental changes around a set concentration of effector (Kascer & Burns, 1973; Kascer & Porteous, 1987).

RESULTS

The $V_{max.}$ appears to be most sensitive to pH changes between 6.8 and 7.1 (mitochondrial pH range), where a 50% decrease in $V_{max.}$ is observed when the pH is decreased from 7.1 to 6.8 (Fig. 1). The K_m values and the K_i/K_m ratios are also markedly changed by changes in pH (Tables 1 and 3). In addition, the 2-oxo acid K_m values increase with increasing pH (Table 1). 3-Methyl-2-oxobutyrate appears to be the 2-oxo acid most sensitive to pH changes, especially between pH 6.8 and 7.3, where the K_m increases 3.2-fold; the K_m for 4-methyl-2-oxopentanoate increases 1.8-fold and the K_m for 3-methyl-2-oxopentanoate increases 1.3-fold between pH 6.8 and 7.3. The change in K_m values between pH 7.3 and 8.0 is less than the change between pH 6.8 and 7.3 for all three 2-oxo acids. The K_m for NAD^+ was measured with and without endogenous dihydrolipoamide dehydrogenase to determine whether or not the exogenously added dihydrolipoamide dehydrogenase alters the value of the kinetic constants determined where NAD^+ is not saturating; however, no differences were found. The K_m for NAD^+ is extremely pH-sensitive, as evidenced by the 7.3-fold decrease in K_m with increasing pH from 6.8 to 8.0 (Table 1). The K_m for CoA is much less sensitive to changes in pH.

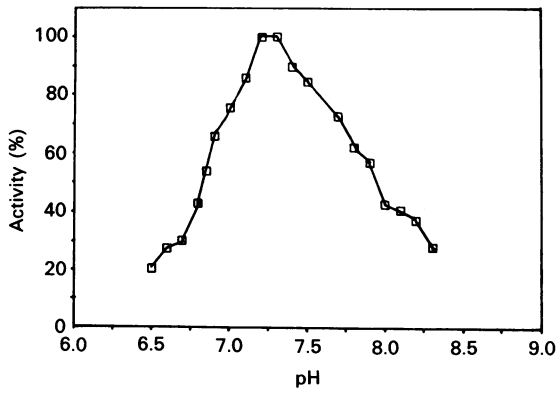

Fig. 1. Effect of pH on maximal BCOADC activity

Assay buffer was prepared as described in the Methods section, and the pH was adjusted with 1 M-KOH or -HCl. All substrates and cofactors were at saturating concentrations, and the reaction was initiated with 3-methyl-2-oxobutyrate.

Table 4. Effect of co-substrate concentration on K_m and K_i values and K_i/K_m ratios for BCOADC at pH 7.3

Kinetic constants were calculated as described in the Methods section.

Competitive inhibitor	[Co-substrate] (mM)	Substrate K_m (μM)	K_i (μM)	K_i/K_m ratio
3-Methylbutyryl-CoA	NAD^+ 1.0	7.4±1.0	10.5±2.0	1.42
	0.125	3.7±0.7	2.5±0.4	0.68
	0.060	1.6±0.4	1.1±0.3	0.69
NADH	CoA 1.0	46.9±5.3	10.2±1.5	0.22
	0.1	49.3±6.5	12.7±2.5	0.26
	0.033	42.7±5.0	9.7±1.0	0.23
	0.017	34.0±6.0	6.7±1.0	0.20

Table 5. Elasticity Coefficients of BCOADC modulators in various tissues

Free effector concentration ratios represent extremes on the high and low ends of previously published values and are thought to represent the physiological ranges of these metabolites. Key to references: [a]LaNoue *et al.* (1972); [b]Kobayashi & Neely (1979); [c]Veech (1978); [d]Baquer *et al.* (1976); [e]Ruderman & Goodman (1973) (from a formula given in Williamson, 1969); [f]Moravec (1980); [g]Taegetmeyer (1984); [h]Siess *et al.* (1977); [i]Ruderman & Goodman (1974); [j]Menahan & Hron (1981). Elasticity Coefficients and increases in [inhibitor]/[substrate] ratios for a 50% decrease in BCOADC activity were calculated from eqn. (1), derived in the Appendix.

Modulator	Tissue	Elasticity Coefficient	Free concentration ratio	Increase in [I]/[S] for a 50% decrease in activity (fold)
NADH/NAD$^+$	Heart	−0.185	0.05[a]	6.4
	Heart	−0.542	0.26[b]	2.8
	Liver	−0.241	0.07[c]	5.1
	Liver	−0.500	0.22[d]	3.0
	Muscle	−0.389	0.14[e]	3.6
Acetyl-CoA/CoA	Heart	−0.074	0.12[f]	14.5
	Heart	−0.157	0.28[g]	7.4
	Liver	−0.074	0.12[d]	14.5
	Liver	−0.167	0.30[h]	7.0
	Muscle	−0.107	0.18[i]	10.3
Acetoacetyl-CoA/CoA	Heart	−0.003	−0.003[j]	369

To determine the potential for feedback inhibition, the kinetic constants for some of the intermediates and end products of branched-chain amino acid oxidation, as well as two fatty acyl-CoA derivatives (palmitoyl-CoA and octanoyl-CoA), were determined (Table 2). To our knowledge, there are few data on the effect of most of these inhibitors on BCOADC activity. All of the acyl-CoA derivatives tested were found to inhibit BCOADC competitively with respect to CoA, except 3-hydroxy-3-methylglutaryl-CoA and succinyl-CoA (3-carboxypropionyl-CoA), which showed no inhibition at concentrations up to 100 μM. At saturating concentrations of co-substrates the K_i values for the acyl-CoAs ranged from 0.2 μM to 17.1 μM, and the K_i/K_m ratios ranged between 0.04 and 1.47. The acyl-CoA present in the branched-chain amino acid catabolic pathway with the lowest K_i was β-methylcrotonyl-CoA, which had a K_i of 5.2 μM. A long-chain fatty acyl-CoA (palmitoyl-CoA) was discovered to have the lowest K_i with respect to CoA (K_i 0.2 μM).

The K_i for NADH was 10.2 μM (at 1 mM-CoA) and the K_i/K_m ratio was 0.22 at pH 7.3 (Table 2). The K_i for NADH decreases slightly as the CoA concentration decreases (Table 4; B. Boyer & R. Odessey, unpublished work), but the K_i/K_m ratio remains constant. The K_i/K_m ratio for NADH was lowest at pH 6.8 where it was 0.02, and increased 11-fold with increasing pH to a value of 0.22 at pH 7.3 (Table 3). The K_i/K_m ratio for 3-methylbutyryl-CoA was much less sensitive to pH, but tended to decrease as the NAD$^+$ concentration decreased.

The Elasticity Coefficients for the acyl-CoAs and NADH are given in Table 5. The values are based on inner mitochondrial metabolite concentrations published in the literature. High and low values for each metabolite concentration are listed for each tissue, and are assumed to encompass a physiological range of concentrations found in the mitochondria of each tissue. Therefore the Elasticity Coefficients represent a range of values corresponding to the range of mitochondrial metabolite concentrations. In addition, we have assumed that the 3-methyl-2-oxobutyrate concentration is approximately equal to its K_m whereas the NAD$^+$ and CoA concentrations are much greater than their K_m values.

Previously published modulator concentrations from various tissues indicate that the mitochondrial free [NADH]/[NAD$^+$] ratio under basal conditions varies from 0.05 to 0.26, depending on the tissue (LaNoue *et al.*, 1972; Baquer *et al.*, 1976), which results in calculated Elasticity Coefficients for NADH of −0.19 to −0.54. Although the K_i/K_m ratio is unaffected by changes in the concentration of CoA (Table 4), the absolute value of the Elasticity Coefficient will decrease if the NAD$^+$ and CoA concentrations in the mitochondria approach the K_m values of NAD$^+$ and CoA respectively.

The mitochondrial [acetyl-CoA]/[CoA] ratio ranges from 0.12 to 0.30 (Baquer *et al.*, 1976; Siess *et al.*, 1977; Moravec, 1980), and thus the calculated Elasticity Coefficient is estimated to be between −0.07 and −0.17. These Elasticity Coefficients are significantly smaller than the Elasticity Coefficients calculated for the NADH/NAD$^+$ couple, because their K_i/K_m ratios are significantly smaller, relative to the [inhibitor]/[substrate] ratio. However, as the NAD$^+$ concentration is decreased, the K_i/K_m ratio is also decreased by about half. This causes the Elasticity Coefficient almost to double, and therefore the inhibitor concentration needed to cause a 50% decrease in BCOADC activity is one-half of the concentration required when NAD$^+$ is saturating. Therefore acetyl-CoA may be a significant inhibitor when the NAD$^+$ concentration is equal to or less than the K_m.

DISCUSSION

To predict the quantitative potential for feedback inhibition of BCOADC activity by NADH, acetyl-CoA and acetoacetyl-CoA, we have calculated their Elasticity Coefficients. When the NAD$^+$ and CoA concentrations are much greater than their K_m values and the 3-methyl-2-oxobutyrate concentration is assumed to be equal to its K_m, a simplified form of the Elasticity Coefficient can be applied (Appendix eqn. 2):

$$\epsilon = \frac{-(K_{m2}/K_{i2}) \times ([I_2]/[S_2])}{\{1 + K_{m1}/[S_1] + (K_{m3}/[S_3]) \times (1 + [I_3]/K_{i3})\} + (K_{m2}/K_{i2}) \times ([S_2]/[I_2])}$$

where S_2 is either NAD^+ or CoA and I_2 is either NADH or R-CoA.

This formula demonstrates that both a low K_i/K_m (high K_m/K_i) ratio and a high [inhibitor]/[substrate] ([I]/[S]) ratio are needed to obtain a large Elasticity Coefficient. This formula illustrates that enzyme activity is much more sensitive to changes in inhibitor concentration when the substrates are saturating. Since co-substrate concentrations were saturating in our experiments, this equation was not used to calculate ϵ in Table 5.

To calculate the Elasticity Coefficient for a competitive inhibitor and infinitesimal changes in modulator concentration, the following formula was derived, based on a similar equation derived by Goldbetter & Koshland (1982) (Appendix eqn. 1), all co-substrates being assumed to be saturating:

$$\epsilon = \frac{-1}{1+\{(K_i/K_m)\times([S]/[I])\}}$$

On the basis of measured values of the mitochondrial [acetyl-CoA]/[CoA] ratio, the Elasticity Coefficient (calculated by eqn. 1) for acetyl-CoA ranged between -0.07 and -0.17 (Table 5). Tischler *et al.* (1977*b*), however, determined that the intramitochondrial [acetyl-CoA]/[CoA] ratio of isolated hepatocyte mitochondria was 1.1:1. On the basis of this concentration ratio the Elasticity Coefficient for acetyl-CoA would be -0.42, and predicts that only a 3.4-fold increase in the [acetyl-CoA]/[CoA] ratio would be required to cause a 50% decrease in BCOADC activity in the liver. These results suggest that conditions such as starvation and diabetes, where the hepatic [acetyl-CoA]/[CoA] ratio has been shown to increase to up to 3 times its basal level (Ruderman & Goodman, 1974; Tischler *et al.*, 1977*a*), may inhibit BCOADC activity. This large a change may be physiologically important because small decreases in flux through BCOADC have been shown to limit branched-chain 2-oxo acid catabolism (Williamson *et al.*, 1986). The effect of acetyl-CoA and other acyl-CoAs in other tissues (e.g. skeletal muscle) require knowledge of the [inhibitor]/[substrate] ratios, which are presently lacking.

The K_m for NAD^+ was 47 μM, which is similar to the value calculated from rat liver (Parker & Randle, 1978*a*), but significantly lower than the K_m found in ox liver (Parker & Randle, 1978*b*). These differences could be due to several factors, for example (1) different substrate concentrations used in the kinetic assays, (2) species- and the tissue-specific differences and (3) purity and stability differences in the enzyme preparations. NADH was found to have a moderately low K_i/K_m ratio (0.22:1) and an Elasticity Coefficient range of -0.18 to -0.54. Thus the redox potential may be an important modulator of BCOADC activity. For example, in the liver 4–5-fold increases in the [NADH]/[NAD^+] ratio have been measured following ischaemia (Veech, 1978), or refeeding after a fast (Wieland *et al.*, 1969), resulting in a decrease in BCOADC activity. It is likely that large changes in the redox potential of other tissues would have similar effects on BCOADC activity. In fact, Williamson *et al.* (1986) have demonstrated that increases in the [NADH]/[NAD^+] ratio or increased acyl-CoA concentration inhibit BCOADC activity in isolated rat liver mitochondria. This further supports the role for feedback inhibition of BCOADC *in vivo*. However, the Elasticity Coefficient only predicts the effect of an increase in [inhibitor]/[substrate] ratio on an isolated enzyme. The modulator may feedback-inhibit other enzymes in the pathway and thus control flux throughout the pathway (Kohn & Chiang, 1982), since a given modulator may act on more than one enzyme (e.g. effect of NADH on other dehydrogenase enzymes distal to BCOADC).

On the basis of the intramitochondrial metabolite concentrations that could be obtained, NADH was calculated to have the highest potential (most negative Elasticity Coefficient) to inhibit BCOADC at its physiological concentration *in vivo*. Both acetyl-CoA and acetoacetyl-CoA, however, could only modulate BCOADC activity *in vivo*, if much larger increases in the [inhibitor]/[substrate] ratio or a decreased NAD^+ concentration existed. Interestingly, regulation of flux through the citric acid cycle is also thought to be primarily controlled by the [NADH]/[NAD^+] ratio at the level of the dehydrogenase reactions (Williamson & Cooper, 1980).

When comparing the acetyl-CoA K_i for PDC with the acetyl-CoA K_i for BCOADC we found that the acetyl-CoA K_i for BCOADC was about 3 times lower than that for PDC, and the K_i/K_m ratio was 3.5 times smaller for BCOADC than for PDC (Roche & Cate, 1977). This would not be expected, since acetyl-CoA is not an immediate end product of the BCOADC reaction, as it is for PDC. The NADH K_i for BCOADC is 30 times lower than the NADH K_i for PDC (Roche & Cate, 1977), but, more importantly, the NADH K_i/K_m ratio for BCOADC was 21 times smaller than the NADH K_i/K_m ratio for PDC. The NADH Elasticity Coefficient for BCOADC is therefore much more negative than the NADH Elasticity Coefficient for PDC (-0.01 to -0.05). Thus, whereas small increases in the [NADH]/[NAD^+] ratio have little direct effect on PDC activity, as evidenced by a small Elasticity Coefficient, most of the effect of NADH on PDC activity is mediated through PDC kinase, as predicted experimentally (Roche & Cate, 1977), and by the computer simulations of the pyruvate-perfused rat heart by Kohn *et al.* (1979*a*). Conversely, the effects of the [NADH]/[NAD^+] ratio and the [acyl-CoA]/[CoA] ratio on the activity of BCOADC kinase appear to be minimal (Paxton & Harris, 1984).

Lastly, the mitochondrial inner-membrane local pH should be considered as a regulatory mechanism for the control of BCOADC activity. As discussed above, the kinetic constants are significantly altered by changes in pH, and enzyme activity is quite sensitive to pH as evidenced by the steep slope of the curve of the plot of pH versus enzyme activity (see Fig. 1). Furthermore, since the mitochondrial inner-membrane local pH is more basic than that of the matrix (Kell & Westerhoff, 1985), small changes in pH could significantly effect the activity of BCOADC. Therefore this pH control mechanism has the ability to alter BCOADC activity with or without feedback inhibitors present.

Studies performed *in vitro* suggest that BCOADC is the major rate-controlling enzyme for branched-chain amino acid degradation. Our control analysis is therefore based on the enzymic properties of purified BCOADC. It will be important to verify the regulatory potential of BCOADC in the control of branched-chain amino acid degradation by determining its Flux Control Coefficient. The Flux Control Coefficient is defined as the change in flux through the pathway for a given change in an enzyme's activity (Kacser & Porteous, 1987). The sum of the Flux Control Coefficients equals unity. In addition, if the Flux Control Coefficient for BCOADC is large, then the Response Coefficient of NADH (product of the Flux Control Coefficient for BCOADC and the Elasticity Coefficient for NADH) will be large. NADH would thus be major regulator of flux through this pathway. Several excellent reviews of metabolic control have been published (Kacser & Burns, 1973; Heinrich & Rapoport, 1974; Kacser & Porteous, 1987).

These studies suggest that BCOADC activity, unlike PDC, may be physiologically regulated by feedback inhibition. Before the Elasticity Coefficients of several of the acyl-CoA derivatives can be determined, it will be necessary to measure their intramitochondrial concentrations. On the basis of their low K_i/K_m ratios they may also significantly contribute to the overall regulation of BCOADC activity by feedback inhibition. The

Elasticity Coefficients for the modulators of BCOADC kinase and BCOADC phosphatase will also need to be determined so that the relative potential for modulation of BCOADC activity by covalent modification can be evaluated and compared with feedback inhibition. This task will be especially difficult since it is not known by what the kinase and phosphatase are controlled. Eventually this treatment should be carried out on all enzymes of the branched-chain 2-oxo acid pathway so that an integrated analysis can be conducted to determine flux control.

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APPENDIX

Derivation of equations

Eqn. (1). Derivation of the Elasticity Coefficient (ϵ) for a competitive inhibitor and infinitesimal changes in modulator concentration

This formula assumes that all co-substrates are saturating. By assuming that the inhibition exerted by these effectors is competitive, the control of BCOADC activity by an end product is given by:

$$v = \frac{V_{max.}}{1+(K_m/[\text{substrate}])\times\{1+([\text{inhibitor}]/K_i)\}}$$

If this expression is differentiated with the use of the formula for Elasticity Coefficient where:

$$\epsilon = \frac{[I]}{v}\times\frac{dv}{d[I]}$$

Then:

$$\epsilon = \frac{-1}{1+K_i/[\text{inhibitor}]\times\{1+([\text{substrate}]/K_m)\}}$$

Furthermore, if $[S]/K_m \gg 1$, then the expression can be simplified to:

$$\epsilon = \frac{-1}{1+(K_i/K_m)\times([S]/[I])}$$

Eqn. (2). Calculation of the Elasticity Coefficient when the substrate and inhibitor concentrations are known

To calculate the Elasticity Coefficient when the substrate and inhibitor concentrations are known (and not necessarily saturating) the following complete rate equation is differentiated to derive the Elasticity Coefficient:

$$\frac{v}{V_{max.}} = \frac{1}{1+K_m/[S_1]+(K_m/[S_2])\times(1+[I_2]/K_i)+(K_m/[S_3])\times(1+[I_3]/K_i)}$$

where S_1–S_3 are the substrates of the reaction, I_2 and I_3 are competitive inhibitors of S_2 and S_3 respectively, and the K_m and K_i values correspond to the substrate or inhibitor by which the kinetic constant is effected.

For the Elasticity Coefficient of I_2 (e.g. NADH):

$$\frac{[I]}{v}\cdot\frac{dv}{d[I]} = \frac{(K_m/[NAD^+])\times([NADH]/K_i)}{A+(K_m/[NAD^+])\times(1+[NADH]/K_i)}$$

where $A = 1+(K_m/[KIV])+(K_m/[CoA])\times(1+[IVC]/K_i)$, KIV and IVC representing 3-methyl-2-oxobutyrate and 3-methylbutyryl-CoA respectively. This can also be written as:

$$\frac{[I]}{v}\cdot\frac{dv}{d[I]} = \frac{(K_m/K_i)\times([NADH]/[NAD^+])}{A+(K_m/[NAD^+])\times(1+[NADH]/K_i)}$$

Eqn. (3). Formula for calculation of the fold change in [inhibitor]/[substrate] ratio for a 50% decrease in BCOADC activity

$$\epsilon = \frac{dv/v}{d[I]/[I]}$$

and for a 50% decrease in activity:

$$-0.5 = \frac{dv}{v}$$

Therefore:

$$-0.5 = (d[I]/[I]) \times \epsilon$$

or

$$d[I]/[I] = 0.5/\epsilon$$

$$-0.5 = \frac{[I]_f - [I]_i}{K_i(1+[S]/K_m)+[I]_f}$$

where $[I]_i$ and $[I]_f$ are the initial inhibitor concentration and the final inhibitor concentration respectiveiy, and solving for $[I]_f/[S]$, where $Q = (1+[S]/K_m)$:

$$0.5Q+0.5[I]_f = [I]_f - [I]_i$$

$$0.5Q+[I]_i = 0.5[I]_f$$

or

$$[I]_f = Q+2[I]_i$$

and

$$[I]_f/[I]_i = (Q+2[I]_i)/[I]_i$$

which is equal to:

$$K_i/[I]_i(1+[S]/K_m)+2$$

Therefore, if $[K]_i/[I]_i \ll 1$:

$$[I]_f/[I]_i = K_i/K_m \times [S]/I_i+2$$

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