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A Mathematical Model of Human Thymidine Kinase 2 Activity

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Abstract

The mitochondrial enzyme thymidine kinase 2 (TK2) phosphorylates deoxythymidine (dT) and deoxycytidine (dC) to form dTMP and dCMP, which in cells rapidly become the negative-feedback end-products dTTP and dCTP. TK2 kinetic activity exhibits Hill coefficients of ~0.5 (apparent negative cooperativity) for dT and ~1 for dC. We present a mathematical model of TK2 activity that is applicable if TK2 exists as two monomer forms in equilibrium.

Keywords

Thymidine kinase 2; mathematical model

INTRODUCTION

The dNTP supply system, critical for DNA replication and repair (1), is important because many anticancer (2) and antiviral (3) nucleoside analogs target or traverse it, and because defects in it can cause mitochondrial DNA depletion syndromes (4). The dNTP supply system has a *de novo* synthesis pathway that has at its core ribonucleotide reductase (RNR: rNDP→dNDP), and a salvage synthesis pathway where deoxynucleosides (dNs) are phosphorylated by deoxycytidine kinase (dCK) and thymidine kinase 1 (TK1) in the cytosol and deoxyguanosine kinase (dGK) and thymidine kinase 2 (TK2) in mitochondria (5). TK2 phosphorylates deoxythymidine (dT; T) and deoxycytidine (dC; C) with Hill coefficients (6) of $h_T = \sim 0.5$ and $h_C = \sim 1$ (7, 8), and its activity is inhibited by its end-products dTTP (t) and dCTP (c), which most likely act as bi-substrate analogs (9) as depicted in Fig. 1 (the single character substrate and inhibitor symbols T, C, t and c will be used in subscripts and equations). TK2 may be a monomer (8), a monomer-dimer mixture (9), or a combination of dimers and tetramers (10, 11). Our recent results suggest that TK2 is predominantly a monomer (Munch-Petersen *et al.* submitted) and we present here a model of TK2 activity data (9) that assumes it exists as two different monomer forms in equilibrium.

MATERIALS AND METHODS

Outlier Removal

The data (9) (Fig. 2) used to estimate model parameters was pared down as follows. In the absence of dNTP inhibition, the k_t profile at $[dC] = 5 \mu\text{M}$ comprised 7% of the data, but it accounted for 33% of the sum of squared errors (SSE), so it was removed; k_t is the specific activity of TK2 as a dT kinase (subscripts on k 's indicate final end-products). Similarly, the k_t profile at $[dTTP] = 1 \mu\text{M}$ was removed because it was 8/114 of the data, but its removal

dropped the SSE from 0.072 to 0.027. Finally, a single k_t point at $[dT] = 161$ and $[dTTP] = 10 \mu\text{M}$ was removed because, out of 106 points, it alone dropped the SSE from 0.027 to 0.025, i.e. less than 1% of the data accounted for 8% of the SSE. These removed outliers are shown as X's in Fig. 2.

Parameter Estimation

The 12-parameter model in Eq. (1) was fitted to the data in Fig. 2 using nonlinear least squares. All model parameters were estimated in exponential forms to constrain parameter estimates and their confidence intervals (CIs) to positive values. Wald CIs were estimated from the Hessian (matrix of second derivatives) of the SSE evaluated at the optimum using the function `optimin R` (12): Hessians were divided by 2, inverted, multiplied by the mean squared error, and the square roots of the main diagonal were then multiplied by 1.96 to form 95% Wald CI.

RESULTS

Model

A graph of our TK2 model as two monomers A and B in equilibrium is presented in Fig. 3. This model assumes TK2 dimers are rare enough to be negligible. Associated with this graph is the following mathematical model of the specific activity of TK2 for its substrates dT and dC:

$$k_t = \frac{k_{t,AT} f \frac{[T]}{K_{AT}}} {1 + \frac{[T]}{K_{AT}} + \frac{[C]}{K_{AC}} + \frac{[d]}{K_{At}} + \frac{[c]}{K_{Ac}}} + \frac{k_{t,BT} (1-f) \frac{[T]}{K_{BT}}} {1 + \frac{[T]}{K_{BT}} + \frac{[C]}{K_{BC}} + \frac{[d]}{K_{Bt}} + \frac{[c]}{K_{Bc}}} \\ k_c = \frac{k_{c,AC} f \frac{[C]}{K_{AC}}} {1 + \frac{[T]}{K_{AT}} + \frac{[C]}{K_{AC}} + \frac{[d]}{K_{At}} + \frac{[c]}{K_{Ac}}} + \frac{k_{c,BC} (1-f) \frac{[C]}{K_{BC}}} {1 + \frac{[T]}{K_{BT}} + \frac{[C]}{K_{BC}} + \frac{[d]}{K_{Bt}} + \frac{[c]}{K_{Bc}}} \quad (1)$$

where f is the fraction of TK2 in monomer form A (i.e. $1 - f$ is the fraction in B), k 's are specific activities, K 's are dissociation constants, and competitive inhibition by dTTP and dCTP (Fig. 1) is assumed. This model has 13 apparent parameters, but only 12 that are identifiable since 5 are found strictly in the 4 parameters $k_{t,AT}f = k_{t,AT}f$, $k_{t,BT}(1-f) = k_{t,BT}(1-f)$, $k_{t,AC}f = k_{c,AC}f$, and $k_{t,BC}(1-f) = k_{c,BC}(1-f)$. These 4 parameters may not be constant since they contain f which could depend on $[dT]$, $[dC]$, $[dCTP]$ and/or $[dTTP]$, but we will assume here that they are; the development of new f measurement methods that will enable f constancy tests is a high priority for this model.

Fit

The fit of Eq. (1) to data in (9) is shown in Fig. 2 and Table 1. Based on parameter estimates in Table 1, form A binds dT more than dC, and form B binds dC more than dT. These differences are qualitatively preserved with triphosphate group additions in the dNTP inhibitors, i.e. form A binds dTTP more than dCTP and form B binds dCTP more than dTTP.

The A form (red in Fig. 4) dominates TK2 dT kinase activity (Fig. 4, left panel) at data points where $[dT]$ is low, and the B form (blue) dominates this activity where $[dT]$ is high. In contrast, no such switch occurs for dC kinase activity (Fig. 4, right panel), i.e. form B dominates this activity entirely. A single monomer dominating the dC kinase activity is consistent with its Hill coefficient being ~ 1 , and A surface shape being similar to dominant B surface shape further conceals the presence of two monomers. We note here that due to this dominance, a Hill coefficient of ~ 1 does not imply equal form A and form B binding constants for dC. Indeed, the confidence intervals of K_{AC} and K_{BC} in Table 1 do not overlap.

In Table 1 $K_{At} = 0.003$ (0.00016, 0.055) μM and $K_{Ac} = 13.8$ (0.54, 354) μM have large upper/lower confidence limit ratios. The reason for this can be gleaned from Fig. 5 which shows the proportion of TK2 activity due to A at data points in Fig. 2, i.e. points to which the model was fitted. This proportion is close to 0 when $[\text{dTTP}] > 0$, and in such cases $[\text{dTTP}] \gg K_{At} = 0.003$ suggests that the data was measured at $[\text{dTTP}]$ too large to estimate K_{At} , though a large amount of data at $[\text{dTTP}] = 0$, being close to 0.003, helped. Meanwhile, for $[\text{dCTP}] > 0$, the proportion of the activity due to A is much higher, and this helps the estimate, but now $K_{Ac} = 13.8 \mu\text{M}$ is further from the data at $[\text{dCTP}] = 0 \mu\text{M}$, rendering it less useful, and with all of the data at $[\text{dCTP}] \leq 5 \mu\text{M}$, $K_{Ac} = 13.8 \mu\text{M}$ is not bracketed by data, so our estimate of it is weak.

DISCUSSION

In the future, mathematical models of TK2 will improve our understanding of how this enzyme works *in vivo* where multiple substrates/inhibitors co-exist. Models of TK2 will also have practical value as components of computer simulations (13) that predict outcomes of TK2 substrate analogs in anticancer and antiviral therapies; a therapeutic-gain conceptual framework for this exists (14). Finally, in mitochondrial depletion syndromes due to TK2 mutations (9), TK2 models might be used to characterize/classify patients: holding the model parameters fixed to wild type values save one at a time, mutant TK2 data could be fitted to find a parameter which represents the mutation best and patients with different mutations but similarly altered TK2 reaction surfaces (perhaps due to the same parameter) might then be treated similarly.

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Figure 1.
TK2 bisubstrate inhibition by dNTP (dCTP or dTTP) binding to the dN pocket.

Figure 2. Fit of model in Eq. (1) to data of Wang *et al* 2003 (9). See Methods regarding deleted outliers X and Table 1 for parameter estimates. Concentrations in the legends are in μM .

Figure 3. Graph of model represented by Eqs. (1). A and B represent two different TK2 monomer forms in equilibrium, C = dC, T = dT, t = dTTP and c = dCTP.

Figure 4.

Predicted dT (left) and dC (right) TK2 kinase activities of the A (red) and B (blue) monomer forms versus $[dT] \times [dC]$ with no inhibition ($[dCTP] = 0$ and $[dTTP] = 0$). Units are $\log_{10} \mu\text{M}$, i.e. $[dN] = 0.1$ to $1000 \mu\text{M}$. The sum of the two forms (gray) fits the data (9) (shown as points, these are the same points as in the upper panels of Fig. 2) well. To avoid $\log(0) = -\infty$ an offset of $10^{-1} \mu\text{M}$ was added to data $[dN]$ values, i.e. points at -1 are actually $[dN] = 0$ values.

Figure 5. Proportion activity due to A at data points in Fig. 2. Legend concentrations are in μM .

Table 1

Parameter estimates for model in Eq. (1).

Parameter	Estimate	Wald 95%.CI
$k_{t,Af}$	0.116	(0.09163, 0.148)
$k_{t,Bf}$	0.489	(0.45521, 0.526)
$k_{c,Af}$	0.152	(0.11765, 0.198)
$k_{c,Bf}$	0.306	(0.28938, 0.326)
K_{AT}	1.112	(0.60110, 2.056)
K_{BT}	17.955	(14.73168, 21.977)
K_{AC}	30.836	(14.73168, 64.715)
K_{BC}	6.200	(5.25931, 7.243)
K_{At}	0.003	(0.00016, 0.055)
K_{Bt}	6.916	(4.48169, 10.697)
K_{Ac}	13.851	(0.54281, 354.249)
K_{Bc}	0.624	(0.46440, 0.837)

Units are 1/sec for k 's and μM for K 's.