A Novel Mathematical Model of Targeted Cancer Therapy along p53 Proteasomal Degradation Pathways

Prem Talwai
Department of Mathematics, American River College, Sacramento, CA, USA
Mira Loma High School, Sacramento, CA, USA

Abstract—Overzealous MDM2-mediated ubiquitination of p53 characterizes and sustains over 50% of all human cancers. Targeted cancer therapy hinges on a thorough understanding of the ubiquitination process. Unfortunately, existing mathematical models inaccurately describe the ubiquitin-proteasome system, due to the underlying assumptions of steady-state and constant cellular concentrations of the ubiquitin-conjugating and ubiquitin ligase enzymes. This paper derives a novel non-steady-state mathematical model of sequential bi-substrate enzyme kinetics, which can be used to simulate the behavioral response of the ubiquitin-proteasome system to specific variations in the cellular concentrations of p53, MDM2, and ubiquitin-conjugated E2D3. From computer simulations of the derived model it was observed that the ubiquitin-ligase MDM2 accelerates the carcinogenic ubiquitination process, while ubiquitin-conjugated E2D3 inhibits it. The mathematical model was also shown to successfully reproduce the experimentally observed p53-MDM2 interaction. The derived model therefore suggests MDM2 as a prospective target for cancer therapy. In addition, the findings of this project propose recombinant E2D3-Ub as a new promising protein-based anticancer drug.

Keywords: targeted cancer therapy, non-steady-state enzyme kinetic model, molecular inhibitor of p53 ubiquitination, protein-based anticancer drug, E2D3

1. Introduction

Overzealous MDM2-mediated ubiquitination of p53 characterizes and sustains over 50% of all human cancers [8]. Successful targeted cancer therapy hinges on a thorough understanding of the ubiquitination process.

The tumor suppressor protein p53, sometimes known as the "guardian of the genome", induces cell cycle arrest in response to cellular stress signals, such as DNA damage or hypoxia. p53 performs its vital function by inducing the transcription of certain genes whose proteins mediate the repair of the genome. Normally only a small amount of p53 actively participates in cellular processes, while the remainder is degraded by the E3 ubiquitin ligase MDM2 [2]. During emergency situations, however, MDM2 is phosphorylated and thus releases p53 to perform its function. A key characteristic of tumor cells is the overexpression of the oncogene MDM2, which results in excessive unregulated degradation of p53 even during abnormal cellular growth.

The crucial process of ubiquitination targets proteins for degradation by attaching chains of ubiquitin molecules to their lysine residues [11]. The process first begins with the activation of ubiquitin by the E1 activating enzyme, using energy in the form of ATP. The ubiquitin then leaves the E1 enzyme and forms a thioester linkage with the cysteine residue of an E2-conjugating enzyme. The latter enzyme transports the ubiquitin to an E3 ubiquitin ligase, which catalyzes the transfer of the ubiquitin (or pre-manufactured polyubiquitin chain) from the E2-conjugating enzyme to the target substrate protein. The ubiquitin binds to the target protein through an isopeptide bond with the substrate’s lysine residue. E3 ubiquitin ligases possess either a HECT (Homologous to the E6-AP Carboxyl Terminus) or RING (Really Interesting New Gene) finger domain for binding with the E2 conjugase. This paper will focus specifically on the kinetics of RING finger ubiquitin ligases.

Unfortunately, existing mathematical models inaccurately describe the ubiquitin-proteasome system, due to the underlying assumptions of steady-state and constant cellular concentrations of the ubiquitin-conjugating and ubiquitin ligase enzymes [3]. The quasi-steady-state assumption underlying existing enzyme kinetic models requires that enzyme concentrations be infinitesimally small in comparison with the concentrations of the substrates [12]. However, in many overzealous ubiquitination processes (such as the degradation of p53), the oncogenic E3 ubiquitin ligase is expressed at much higher levels than the target substrate protein. This unique characteristic of many cancerous systems invalidates the quasi-steady-state assumption and therefore prohibits the use of current enzyme kinetic models.

In addition, current in vitro studies solely report the behavior of the ubiquitin-proteasome system at constant concentrations of the E3 ligase and ubiquitin-conjugated E2 [9]. However in actual cancer cells, the concentrations of these ubiquitination enzymes fluctuate considerably. Therefore, the results of such in vitro studies fail to accurately describe true protein ubiquitination. Furthermore, current multi-substrate enzyme kinetic models solely analyze the highly uncontrollable relationships between substrate activity and product formation rate during the course of enzyme catalysis. However, in most chemical reactors, the initial concentrations of
the enzymes, substrates, and products are more easily varied in order to achieve optimal system performance.

This paper derives a novel non-steady-state mathematical model of sequential bi-substrate enzyme kinetics, which can be used to simulate the behavioral response of the ubiquitin-proteasome system (UPS) to specific variations in the cellular concentrations of targeted p53, MDM2, and ubiquitin-conjugated E2D3. The derived mathematical model may be used to analyze the effects of specific modifications in the initial conditions on the reaction progress curves for the enzyme, substrates, and products. Computational simulations of the derived mathematical model are provided in order to illustrate the effects of MDM2 and E2D3-Ub concentrations on the rates of multiple p53 ubiquitination processes (each generated by a different set of rate constants). The paper concludes with a detailed description of the implications of the aforementioned computational results on targeted cancer therapy and drug discovery.

2. Materials and Methods

MDM2-mediated ubiquitination of p53 follows a compulsory-order ternary complex mechanism [13]. The target protein p53 and the ubiquitin-conjugated E2D3 bind the E3 ligase MDM2, which transforms the latter substrates into their corresponding products (one of which is ubiquitinated p53). The general sequential bi-substrate enzyme mechanism can be summarized as follows:

\[
\begin{align*}
A + B & \overset{k_1}{\rightarrow} C_1 + B \\
C_1 + B & \overset{k_2}{\rightarrow} C_2 \\
C_2 & \overset{k_3}{\rightarrow} C_3 \\
C_3 & \overset{k_4}{\rightarrow} C_4 + P \\
C_4 + P & \overset{k_5}{\rightarrow} E + P + Q
\end{align*}
\]

where \( A \) and \( B \) are p53 and E2D3-Ub respectively and \( E \) is the RING finger enzyme MDM2. If we let \( C_1, C_2, C_3, \) and \( C_4 \) be the complexes \( EA, EAB, EPQ, \) and \( EQ \), then the mechanism may be summarized by the following set of elementary reactions:

\[
E + A + B \overset{k_1}{\rightarrow} C_1 + B \\
C_1 + B \overset{k_2}{\rightarrow} C_2 \\
C_2 \overset{k_3}{\rightarrow} C_3 \\
C_3 \overset{k_4}{\rightarrow} C_4 + P \\
C_4 + P \overset{k_5}{\rightarrow} E + P + Q
\]

2.1 Construction of Model (System of Partial Differential Equations)

The Law of Mass Action was used to derive a nonlinear autonomous system of differential equations to model the reaction mechanism. Upon close investigation of this system of ODEs the following relationships were discovered:

\[
\begin{align*}
\frac{dE}{dt} &= \frac{dA}{dt} + \frac{dQ}{dt} \\
\frac{dC_2}{dt} + \frac{dC_3}{dt} &= -\left( \frac{dB}{dt} + \frac{dP}{dt} \right) \\
\frac{dC_1}{dt} &= \frac{dB}{dt} - \frac{dA}{dt} \\
\frac{dC_4}{dt} &= \frac{dP}{dt} - \frac{dQ}{dt}
\end{align*}
\]

The concentrations of the various species throughout the reaction were allowed to depend not only on the time but also on the initial concentrations of the enzyme and substrates. This novel modification enables scientists to use the derived model for the investigation of precisely how the rates of certain enzyme-catalyzed reactions are affected by specific variations in the cellular concentrations of the enzymes and substrate proteins. By integrating both sides of the equations in (1), the concentrations of the enzyme and the intermediate complexes were expressed in terms of the concentrations of the substrates, products, and four unknown functions of the initial conditions. In the following derivations, let the variable \( s \) = the initial concentration of substrate \( A \), the variable \( u \) = the initial concentration of substrate \( B \), the variable \( v \) = the initial concentration of enzyme \( E \), and the parameter \( P_0 \) = the initial concentration of the product \( P \).

In order to determine the four unknown functions, the uncatalyzed reaction corresponding to the ordered bi-substrate enzyme mechanism was first analyzed. The substrate and product concentrations were then expressed in terms of the initial conditions and the extent of reaction. In addition, upon detailed comparison with the catalyzed reaction, the various states in which the substrates and products existed during the course of enzyme catalysis were discovered. It was noticed that the substrate \( A \) occurred in three states (a free state \( A \) and two bound states \( C_1 \) and \( C_2 \)), the substrate \( B \) occurred in two states (a free state \( B \) and one bound state \( C_2 \)), the product \( P \) occurred in two states (a free state \( P \) and one bound state \( C_3 \)), and finally the product \( Q \) occurred in three states (a free state \( Q \) and two bound states \( C_3 \) and \( C_4 \)). The total substrate and product concentrations were expressed as the sum of the concentrations of each of their respective free and bound states.

Using these derived relationships, the four unknown functions were determined and the concentrations of the product \( Q \) and intermediate complexes \( C_1, C_3, C_4 \) were precisely expressed in terms of the concentrations of the enzyme, substrates, complex \( C_2 \), product \( P \), and the initial conditions. The equations were as follows:

\[
\begin{align*}
Q &= E - A - v + s \\
C_1 &= B - A + s - u \\
C_3 &= u + P_0 - (P + B + C_2) \\
C_4 &= P - E + A + v - s - P_0
\end{align*}
\]
The equations in (2) were then substituted into the original system of nine ODEs. This substitution enabled the elimination of four variables from the original system which rapidly simplified the computational simulation of the model and eliminated the need for inaccurate steady-state or constant-concentration assumptions. In addition, this substitution enabled the introduction of novel initial concentration variables into the mathematical model. The resulting nonlinear system of partial differential equations was as follows:

\[
\frac{\partial E}{\partial t} (s, t, u, v) = k_5 (P - E + A + v - s - P_0) - k_1 EA \tag{3a}
\]

\[
\frac{\partial A}{\partial t} (s, t, u, v) = -k_1 EA \tag{3b}
\]

\[
\frac{\partial B}{\partial t} (s, t, u, v) = -k_2 B (B - A + s - u) \tag{3c}
\]

\[
\frac{\partial C_2}{\partial t} (s, t, u, v) = k_3 B (B - A + s - u)
+ k_{-3} (u + P_0 - (P + B + C_2))
- k_3 C_2 \tag{3d}
\]

\[
\frac{\partial P}{\partial t} (s, t, u, v) = k_4 (u + P_0 - (P + B + C_2)) \tag{3e}
\]

Using the original system of ODEs and the relationships in (2), an equilibrium point for the nonlinear system in (3) was found to be \((v, 0, u - s, 0, s + P_0)\). The nonlinear system of PDEs was subsequently linearized about this equilibrium point and the following linear system was obtained:

\[
\bar{V}' = J (v, 0, u - s, 0, s + P_0) \bar{V} \tag{4}
\]

with \(\bar{V}' = \begin{bmatrix} E - v & A & B + s - u & C_2 & P - P_0 - s \\ 0 & s & 0 & -s \end{bmatrix} J(v, 0, u - s, 0, s + P_0), \) the Jacobian of the nonlinear system at the equilibrium point, is given by:

\[
\begin{bmatrix}
-k_5 & -k_1 v + k_5 & 0 & 0 & k_5 \\
-0 & k_1 v - k_2 s & 0 & 0 & 0 \\
0 & k_2 u - k_2 s & k_2 s - k_2 u & 0 & 0 \\
0 & k_2 s - k_2 u & k_2 u - k_2 s - k_{-3} & -k_{-3} - k_3 & -k_{-3} \\
0 & 0 & -k_4 & -k_4 & -k_4
\end{bmatrix}
\]

### 2.2 Solution to Model

The perturbed linear system was subsequently solved using conventional linear algebraic techniques. From the eigenvalues of the Jacobian \(J(v, 0, u - s, 0, s + P_0)\), the equilibrium \((v, 0, u - s, 0, s + P_0)\) was found to be asymptotically stable for all biologically relevant conditions. The perturbations in each of the five variables were expressed as linear combinations of five exponential functions of time (whose growth “constants” depended on the initial conditions). The coefficients of these linear combinations (which were functions of the initial concentration variables) were determined through large-scale row reduction using the Mathematica program. In the interest of space, an abbreviated version of the final set of integrated rate laws will be provided in the results section. The complete model is incorporated into the computational simulations.

### 3. Results

The final mathematical model of the sequential bi-substrate enzyme mechanism was:

\[
[MDM2] = n_1 (s, u, v) e^{\lambda_1 t} + n_2 (s, u, v) D_1 + D_2 e^{\lambda_2 t} \tag{5a}
\]

\[
+ n_3 (s, u, v) C_{13} e^{\lambda_3 t} + n_4 (s, u, v) C_{15} e^{\lambda_5 t} + n_5 (s, u, v) C_{16} e^{\lambda_6 t} + v
\]

\[
[p53] = n_2 (s, u, v) D_5 + D_6 e^{\lambda_2 t} \tag{5b}
\]

\[
[E2D3 - Ub] = n_2 (s, u, v) C_{27} v^3 + C_{28} v^2 + C_{29} v + C_{30} e^{\lambda_3 t} \tag{5c}
\]

\[
[C_2] = n_2 (s, u, v) (C_{35} v^2 + C_{36} v) e^{\lambda_4 t} + n_3 (s, u, v) D_8 e^{\lambda_5 t} + u - s \tag{5d}
\]

\[
[p53 - Ub] = n_2 (s, u, v) e^{\lambda_2 t} + n_3 (s, u, v) e^{\lambda_3 t} + n_4 (s, u, v) e^{\lambda_5 t} + n_5 (s, u, v) e^{\lambda_6 t} + P_0 + s \tag{5e}
\]

where \(D_1, D_2, \ldots, D_9\) are functions of \(s, u\) and \(v\) given by:

\[
D_1 = C_1 v^5 + C_2 v^4 + C_3 v^3 + C_4 v^2 + C_5 v + C_6 u v^4 + C_7 u v^3 + C_8 u v^2 \tag{6a}
\]

\[
D_2 = C_9 u v - C_6 u v^4 - C_7 u v^3 \tag{6b}
\]

\[
D_3 = C_{10} u v + C_{11} u - C_{10} u v^2 \tag{6c}
\]

\[
D_4 = C_{12} u v + C_{11} u - C_{12} u v^2 \tag{6d}
\]

\[
D_5 = C_{17} v^4 + C_{18} v^3 + C_{19} v^2 + C_{20} v + C_{21} u v^3 + C_{22} u v^2 \tag{6e}
\]

\[
D_6 = C_{23} u v + C_{24} u - C_{23} u v^3 - C_{22} u v^2 \tag{6f}
\]

\[
D_7 = C_{25} u v + C_{26} u - C_{25} u v - C_{26} \tag{6g}
\]

\[
D_8 = C_{32} u^2 + C_{33} u^2 - 2 C_{32} u + C_{33} \tag{6h}
\]

\[
D_9 = -C_{32} u^2 - C_{33} u^2 + 2 C_{32} u - C_{33} \tag{6i}
\]

\[
\lambda_1, \lambda_2, \ldots, \lambda_5 \text{ are the eigenvalues of } J(v, 0, u - s, 0, s + P_0). \]

The functions \(n_1 (s, u, v), n_2 (s, u, v), \ldots, n_5 (s, u, v)\) are omitted from this abbreviated model due to their length and complexity. Lengthy nonlinear expressions involving the rate constants are replaced by kinetic constants of the form \(C_i\) in order to aid parameter estimation (definitions of \(C_i\) and the aforementioned coefficient functions are incorporated into the computer simulations; definitions of \(C_i\) can also
be found in Table 1). Note that in (5) the generic variables for the enzyme, substrates, and product have been replaced by the specific proteins that perform their functions in the process of p53 ubiquitination.

3.1 Computer Simulations of Mathematical Model

The mathematical model was simulated for several p53 ubiquitination processes by varying the values of the reaction rate constants (all given in min⁻¹). A detailed explanation and graphical representation of three such simulations is provided in Figure 2. From Figure 2, it is quite evident that the substrate E2D3-Ub inhibits overzealous p53 ubiquitination while the E3 ligase MDM2 accelerates this carcinogenic reaction. In addition, it can be seen that E2D3-Ub is a more effective inhibitor of p53 ubiquitination when present at higher concentrations (the curves are steepest in the rightmost cluster of each substrate-velocity plot). However, the simulations also show that high concentrations of p53 can hinder the ability of E2D3-Ub to decelerate the reaction (an increase in the initial concentration of p53 makes the substrate-velocity curves more gradual).

3.2 Agreement with Experimental Results

The model accurately reproduces the experimentally observed p53-MDM2 interaction reported in various in vitro studies [7,5,1]. For example, in [10] it was observed that wild-type p53 ubiquitination increased rapidly when the MDM2 protein was overexpressed. The experimentally observed p53-MDM2 interaction was primarily reported qualitatively through Western Blot analysis. Since an ordered bi-bi mechanism produces both free and bound substrate and product states, distinction between the optical bands corresponding to these different states and subsequent densitometric quantification of the western blots becomes difficult [4]. Therefore empirical substrate-velocity curves for p53 ubiquitination were not obtainable for direct comparison against the derived model and an alternative method, known as sensitivity analysis [6], was used for model validation (please refer to Figure 3 for details).

4. Conclusion

Computer simulations of the derived model identify MDM2 as a potential target for cancer therapy and demonstrate that E2D3-Ub exhibits antitumor activity. Recombinant forms of E2D3-Ub may therefore function as a promising protein-based anticancer drug for targeting overzealous p53 ubiquitination. The derived model’s successful duplication of the experimentally observed p53-MDM2 interaction confirms its validity in simulating less explored biochemical relationships, such as that between p53 and E2D3-Ub. Further in vitro experimentation is obviously necessary for additional verification of the therapeutic properties of ubiquitin-conjugated E2D3.

The non-steady-state mathematical model derived in this paper may be used for the kinetic analysis of various biochemical processes governed by ordered ternary-complex mechanisms, most notably the synthesis of DNA molecules by DNA polymerase, the detoxification of lipophilic xeno-biotics by glutathione S-transferase, and the oxidation of NADH to NAD⁺ by L-lactate dehydrogenase. Extrapolation of the model can be used to identify the most favorable initial conditions for generating optimal performance of many commercial chemical reactors. The findings of this paper may also be employed to determine treatment solutions for several other bacterial infections and inflammatory diseases (such as rheumatoid arthritis) characterized by an overzealous UPS. The model can be utilized for the discovery of new techniques to accelerate healthy biological processes and inhibit harmful protein activity. Finally, computational simulation of the derived model provides a safe, fast, and cost-effective preliminary alternative to expensive in vitro experimentation.

References

Fig. 2: Each column features a different simulation of the mathematical model. In each column, the top figure shows the influence of E2D3-Ub concentration on p53 ubiquitination rate for various initial concentrations of p53 and E2D3-Ub. The three distinct clusters of curves correspond to three different initial concentrations of E2D3-Ub: 5 µM, 7.5 µM, 10 µM. In each cluster, the dashed curves approach the thick curve as the initial concentration of p53 is increased from 1 µM to 3 µM (in increments of 0.5 µM) while the initial concentration of MDM2 is held fixed at 20 µM. The bottom figure shows the influence of total (initial) MDM2 concentration on p53 ubiquitination rate at different moments during the reaction. Note that in each simulation, the influence of E2D3-Ub concentration on ubiquitination rate was only investigated during the period of time before the substrate E2D3-Ub settled at a relatively steady concentration. The parameter values used to generate these simulations were: (Left) $k_1 = 0.15$ µM, $k_2 = 0.175$ µM, $k_3 = 0.125$ µM, $k_{-3} = 0.1$ µM, $k_4 = 0.2$ µM, $k_5 = 0.25$ µM (Center) $k_1 = 0.25$ µM, $k_2 = 0.275$ µM, $k_3 = 0.225$ µM, $k_{-3} = 0.2$ µM, $k_4 = 0.3$ µM, $k_5 = 0.35$ µM (Right) $k_1 = 0.35$ µM, $k_2 = 0.375$ µM, $k_3 = 0.325$ µM, $k_{-3} = 0.3$ µM, $k_4 = 0.4$ µM, $k_5 = 0.45$ µM.

Fig. 3: Sensitivity analysis is used here to describe how the model responds to drastic changes in the input parameters. The rate constants used to generate the leftmost column of Figure 2 were modified by various powers of 10, and the effect on the simulated behavior of the p53 ubiquitination system was studied. It was observed that MDM2 continued to accelerate the ubiquitination process while E2D3-Ub persistently worked to inhibit it. Simulations for a modification by a factor of (Left) $10^5$ and (Right) $10^6$ are provided.
### Table 1: Definitions of Model Parameters

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<th>Constants</th>
<th>Definitions</th>
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<td>$-\frac{2k_{-3}}{k_{-3} + k_3 - k_4 - \sqrt{-4k_3k_4 + (k_{-3} + k_3 + k_4)^2 + 2k_5}}$</td>
</tr>
</tbody>
</table>