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A mathematical model for apoptosome assembly: The optimal cytochrome c/Apaf-1 ratio

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Contents

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Abstract

Apoptosis, a highly conserved form of cell suicide, is regulated by apoptotic signals and their transduction with caspases, a family of cystein proteases. Caspases are constantly expressed in the normal cells as inactive pro-enzymes. The activity of caspase is regulated by the proteolysis. Sequential proteolytic reactions of caspases are needed to execute apoptosis. Mitochondrial pathway is one of these apoptotic signal pathways, in which caspases are oligomerized into characteristic heptamer structure, called apoptosome, with caspase-9 that activate the effector caspases for apoptosis. To investigate the dynamics of signal transduction pathway regulated by oligomerization, we construct a mathematical model for Apaf-1 heptamer assembly process. The model first reveals that intermediate products can remain unconverted even after all assemble reactions are completed. The second result of the model is that the conversion efficiency of Apaf-1 heptamer assembly is maximized when the initial concentration of cytochrome c is equal to that of Apaf-1. When the concentration of cytochrome c is sufficiently larger or smaller than that of Apaf-1, the final Apaf-1 heptamer production is decreased, because intermediate Apaf-1 oligomers (tetramers and bigger oligomers), which themselves are unable to form active heptamer, accumulate too fast in the cells, choking a smooth production of Apaf-1 heptamer. Slow activation of Apaf-1 monomers and small oligomers increase the conversion efficiency. We also study the optimal number of subunits comprising an active oligomer that maximize the conversion efficiency in assembly process, and found that the tetramer is the optimum.

Keywords: apoptosis, apoptosome, mitochondrial pathway, cytochrome c, Apaf-1, oligomerization

1 Introduction

Apoptosis is a highly conserved form of cell suicide, which plays a key role in controlling the cell number in multicellular organisms(Zimmermann *et al.*, 2001). The apoptotic signals are transduced through the specific pathways. All of the apoptotic signal pathways are composed of the cascade of caspase reactions. Caspases are highly conserved cystein protease(Nunez *et al.*, 1998) that are found in variety of multicellular organisms. In human, 14 caspases are identified. Caspases play critical roles in regulating apoptosis and inflammation of cells, and are constantly expressed in normal cells as inactive pro-enzymes. The activity of caspases is strictly regulated by the protein cleavage. Once caspase is cleaved into subunits, an active complex is produced from the large and the small subunits. The active complex of caspase can cleave the specific site of caspase and/or substrates in the downstream of the cascade. The apoptotic signal is transduced by this sequential activations of caspases. Caspases are divided into two classes by their functions in this cascade. One, called initiator caspase, takes part in the upstream of the cascade, which are activated by the receptor of the apoptotic signal and/or the abnormality of the cells such as DNA damages. The other, called effector caspase, takes part in the downstream of the cascade, which is cleaved and activated by the initiators. Effector caspases can cleave and activate many other substrates that mediate the apoptosis.

There are two major pathways of apoptosis – extrinsic triggering ("death receptor mediated) pathways and intrinsic triggering (by stress, chemotherapeutics, and UV irradiation) pathways(Scaffidi *et al.*, 1998). In the receptor mediated pathway, the ligands such as tumor necrosis factor bind to the specific "death receptor, initiating the apoptosis of the cell as extrinsic stimuli. These receptors are characterized by the conserved cytoplasmic sequence called the death domain. The ligand and receptor complex can bind to caspase-8 through the adaptor molecule FADD. FADD links between the ligand/receptor complex and caspase-8. Caspase-8 recruited by the FADD is activated by auto cleavage. Activated caspase-8 can cleave and activate the effector caspase-3 and/or

-7 directly.

The intrinsic stimuli such as stress, chemotherapeutics and UV irradiation induces the release of cytochrome c from the mitochondria, which activates Apaf-1. In the present paper, we focus on the process of the activation of Apaf-1 monomers and their assembly to heptamers in the intrinsic triggering pathway of apoptosis.

Either Bid truncation by active caspase-8 or the abnormality of cells triggers the release of the cytochrome c from mitochondria to cytoplasm(Jiang & Wang, 2004; Sprick & Walczak, 2004). The released cytochrome c activates the Apaf-1 (Apoptotic Protease Activating Factor-1) by introducing the conformational change of Apaf-1. Activated Apaf-1 monomers then interact with each other to form Apaf-1 oligomers. This oligomerization of Apaf-1 monomers , and subsequent oligomerization of intermediate Apaf-1 oligomers, finally gives rise to a 700kDa large protein complex called apoptosome(Cain *et al.*, 1999; Zou *et al.*, 1999; Saleh *et al.*, 1999; Cain *et al.*, 2000; Adams & Cory, 2002; Cain *et al.*, 2002). Caspase-9 is activated without cleaving them in apoptosome. Unlike other caspases, the activity of caspase-9 is regulated, not by its cleavage by upstream caspases, but by the apoptosome produced through the oligomerization of activated Apaf-1's. The schematic illustration of mitochondrial pathway is shown in Fig. 1. The alteration of the factors regulating the apoptosome assembly contribute to pathogenesis of the cancer(Hajra & Liu, 2004). Apoptosome acts as an executioner of the apoptosis.

Cells are divided into two classes by their choice of the primary apoptotic pathways(Scaffidi *et al.*, 1999). Type I cells are characterized by their greater amount of death-inducing signaling complex (DISC), which is composed of death receptor and adapter protein FADD, than type II cells. In type I cells, caspase-8 is recruited and activated by the DISC within seconds and is caspase-3 in 30 minutes. Whereas, in type II cells, the recruitment of FADD and caspase-8 by death receptor is strongly suppressed. Caspase-3 are primarily activated by caspase-9 through the mitochondrial pathway in type II cells. The activation of caspase-8 and caspase-3 takes 60 minutes in type II

cell. Because apoptosome is a large protein complex which are formed through complex protein interactions, it is difficult to investigate the dynamics of the apoptosome assembly experimentally. It is not yet clear how the activated Apaf-1 oligomers interact with each other to make Apaf-1 heptamer. We therefore highlight the process of Apaf-1 heptamer assembly by using a simple mathematical model.

Previous mathematical models on the apoptotic signal transduction proposed so far(Fussenegger *et al.*, 2000; Bentele *et al.*, 2004) have handled the process of apoptosome assembly as a single non-linear reaction term, where complex interactions between oligomers (from monomer to heptamer) are embedded into a nonlinearity parameter. It is important, however, to develop a model for the oligomerization of Apaf-1 itself, because the accuracy and swiftness of apoptosis critically depends on how efficient and quickly the Apaf-1 heptamer is assembled. We here construct a simple mathematical model of Apaf-1 heptamer assembly, and study how the oligomerization process of Apaf-1's is adjusted for an efficient regulation of the activity of caspase-9, the key molecule that finally switches on the apoptotic reaction.

2   A model for Apaf-1 heptamer assembly

We here consider the formation of large protein complexes that are produced by the pairwise assembly among two or more kinds of unit proteins. We focus on the oligomerization of Apaf-1, but the result would be applicable for other system with similar assembly process. We first consider the activation of Apaf-1 monomer. Cytochrome c in the cytoplasm binds to an inactive Apaf-1 and changes its conformation(Acehan *et al.*, 2002). Apaf-1 is 130kDa protein that contains two WD-40 repeats in the C-terminal side and CARD (CAspase Recruitment Domain) in the N-terminal side(Vaughn *et al.*, 1999). In inactive Apaf-1, CARD domain binds WD-40 repeat of Apaf-1. Cytochrome c interacts with Apaf-1 through the WD-40 repeats. The Apaf-1 then changes its conformation by extending its CARD domain towards outside. Activated Apaf-1 with extended

CARD domain can interact with each other through its CARD domain(Shiozaki *et al.*, 2002). We assume that cytochrome c is released from mitochondria within a sufficiently short time. The release of cytochrome c is triggered by the abnormality of cells, and regulated by caspase-8 which is initiator of apoptotic signal transduction. By denoting by α the association constant for the binding of cytochrome to Apaf-1, the reaction formula is

$$\text{cytochrome } c + \text{inactive Apaf-1} \xrightarrow{\alpha} \text{active Apaf-1}$$

We denote the concentration of cytochrome c and inactive Apaf-1 by c and y, respectively. The time change of cytochrome c and inactive Apaf-1 concentrations is then given by

$$\frac{dc}{dt} = \frac{dy}{dt} = -\alpha y c \tag{1}$$

The activated Apaf-1 can interact with each other with association rate β. The 3-D structure of apoptosome(Acehan *et al.*, 2002) is revealed that apoptosome is composed of 7 Apaf-1 molecules. We consider the assembly of two Apaf-1 oligomers for the mathematical simplicity, one with i Apaf-1 molecules and another with j molecules, giving rise to a larger oligomer composed of $i+j$ Apaf-1's, but only when $i + j$ is less than 7. An intermediate oligomer dissociates into smaller oligomers again with the dissociation constant γ. The diagram of oligomerization is shown in Fig. 2. The reaction formula for this is

$$\text{oligomer}_i + \text{oligomer}_j \quad \underset{\gamma}{\overset{\beta}{\rightleftharpoons}} \quad \text{oligomer}_{i+j} \tag{2}$$

The concentration of Apaf-1 oligomer composed of i molecules of Apaf-1 is designated by x_i. The time change of the concentration of each oligomer is given from both the term of the production and the consumption. The active Apaf-1 monomer is produced from inactive Apaf-1 and can interact with oligomers composed of 1 to 6 Apaf-1 molecules. The time change of Apaf-1

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active monomer is then given as

$$
\begin{aligned}
\frac{dx_1}{dt} &= \alpha yc - \beta x_1(2x_1 + x_2 + x_3 + x_4 + x_5 + x_6) + \gamma(2x_2 + x_3 + x_4 + x_5 + x_6 + x_7) \\
\frac{dx_2}{dt} &= \beta x_1^2 - \beta x_2(x_1 + 2x_2 + x_3 + x_4 + x_5) + \gamma(x_3 + 2x_4 + x_5 + x_6 + x_7) - \gamma x_2 \\
\frac{dx_3}{dt} &= \beta x_1 x_2 - \beta x_3(x_1 + x_2 + 2x_3 + x_4) + \gamma(x_4 + x_5 + 2x_6 + x_7) - \gamma x_3 \\
\frac{dx_4}{dt} &= \beta(x_1 x_3 + x_2^2) - \beta x_4(x_1 + x_2 + x_3) + \gamma(x_5 + x_6 + x_7) - 2\gamma x_4 \\
\frac{dx_5}{dt} &= \beta(x_1 x_4 + x_2 x_3) - \beta x_5(x_1 + x_2) + \gamma(x_6 + x_7) - 2\gamma x_5 \\
\frac{dx_6}{dt} &= \beta(x_1 x_5 + x_2 x_4 + x_3^2) - \beta x_6 x_1 + \gamma x_7 - 3\gamma x_6 \\
\frac{dx_7}{dt} &= \beta(x_1 x_6 + x_2 x_5 + x_3 x_4) - 3\gamma x_7
\end{aligned}
\tag{3}
$$

Note that though we have modeled the dissociation process as the reverse reaction of the association of two molecules (see (2)), there might be other ways to formulate dissociation (e.g. collapse of a n-mer into n monomers at once). There seems to be no empirical evidence for or against our assumption in dissociation process.

Though actual apoptosomes are composed of 7 Apaf-1 molecules, the model can be expanded to the cases where the final product is composed of n molecules. Active monomer can then be assembled with oligomers composed of 1 to $n-1$ molecules. Likewise the oligomer composed of i molecules can be assembled with oligomers composed of j molecules, (but only when $i+j$ is less than n). For mathematical simplicity, we first examined the Apaf-1 heptamer assembly without any dissociation reaction ($\gamma = 0$). We then examined the effect of the positive dissociation constant later. Throughout the paper, we used the reported association constant of interaction between cytochrome c and Apaf-1: $\alpha = 0.004$ ($\mu M^{-1}s^{-1}$)(Purring-Koch & McLendon, 2000). The time

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change of the concentration of each oligomers is then given as

$$
\begin{aligned}
\frac{dx_1}{dt} &= \alpha y c - \beta x_1 \sum_{j=1}^{n-1}(1 + \delta_{1j})x_j \\
\frac{dx_i}{dt} &= \beta \sum_{k=1}^{[i/2]} x_k x_{i-k} - \beta x_i \sum_{j=1}^{n-i}(1 + \delta_{ij})x_j \\
\frac{dx_n}{dt} &= \beta \sum_{k=1}^{[n/2]} x_k x_{n-k}
\end{aligned}
\tag{4}
$$

where $[n/2]$ denotes the largest integer which is less than or equal to $n/2$, and $\delta_{ij} = 1$ if $i = j$ and $\delta_{ij} = 0$ if $i \neq j$. The initial concentration of cytochrome c and Apaf-1 is denoted by c_0 and y_0, respectively. Other initial densities are assumed to be zero: $x_i(0) = 0$, $(i = 1, 2, \cdots, n)$.

To non-dimensionalize the equations (4) with $\gamma = 0$, we denote the concentration $x_i(t)$, $y(t)$ and $c(t)$ relative to that y_0 of the initial inactive Apaf-1 by $X_i(t) = x_i(t)/y_0$, $Y(t) = y(t)/y_0$ and $C = c(t)/y_0$, respectively. Next we rescale time as $\tau = \alpha y_0 t$. The model (1) and (4) in terms of X_i, Y, C and τ are expressed as

$$
\begin{aligned}
\frac{dC}{d\tau} &= \frac{dY}{d\tau} = -YC \\
\frac{dX_1}{d\tau} &= YC - \lambda X_1(2X_1 + X_2 + \cdots + X_{n-1}) \\
\frac{dX_i}{d\tau} &= \lambda \left[\sum_{k=1}^{[i/2]} X_k X_{i-k} - X_i \sum_{j=1}^{n-i}(1 + \delta_{ij})X_j \right] \\
\frac{dX_n}{d\tau} &= \lambda \sum_{k=1}^{[n/2]} X_k X_{n-k}
\end{aligned}
\tag{5}
$$

where $\lambda = \beta/\alpha$. The initial conditions are $X_i(0) = 0, (i = 1, 2, \cdots, n), Y(0) = 1$, and $C(0) = C_0 = c_0/y_0$. There are only two parameters, λ and C_0 in the rescaled dynamics. An example of the time course of the concentration of each oligomer is shown in Fig. 3.

3   The equilibrium concentrations of intermediate and final oligomers

Now we consider the ratio of the equilibrium concentration of the final product to that of initial inactive Apaf-1. Because it is reported that the stoichiometry of Apaf-1 to procaspase-9 is 1:1, 7 molecules of caspase-9 are required to saturate binding sites on the apoptosome composed of 7 Apaf-1 molecules. The local high concentration of procaspase-9 in apoptosome may facilitate to form active dimer of caspase-9. It is very important to examine the number of Apaf-1 molecules contained in final product of apoptosome assembly. When the concentration of the final product becomes $1/7$ of inactive Apaf-1, the efficiency of the conversion of the Apaf-1 heptamer assembly becomes 100%. We define the conversion efficiency as the ratio of the equilibrium concentration $x_n(\infty)$ of the final product to $1/n$ of the initial concentration y_0 of inactive Apaf-1: i.e. $nX_n(\infty)$. We first focus on the equilibrium after a sufficiently long time has passed since the introduction of the apoptotic signal. As shown in section 6, the result without dissociation when sufficiently long time has passed (large a t) compatible with the result with dissociation when γ is sufficiently small. Because cytochrome c and inactive Apaf-1 is only consumed by the interaction with each other, $\hat{Y}\hat{C} = 0$. From the equilibrium condition for the time change of active monomer is described as concentration

$$\hat{X}_1(2\hat{X}_1 + \cdots + \hat{X}_{n-1}) = 0,$$

we immediately have $\hat{X}_1 = 0$. Similarly, from the equilibrium condition for $\hat{X}_i$, we have

$$\sum_{k=1}^{[i/2]} \hat{X}_k \hat{X}_{i-k} - \hat{X}_i \sum_{j=1}^{n-i}(1 + \delta_{ij})\hat{X}_j = 0.$$

Suppose now that $\hat{X}_1 = \hat{X}_2 = \cdots = \hat{X}_{i-1} = 0$. It then follows that, if $i \leqslant [n/2]$,

$$\sum_{k=1}^{[i/2]} \hat{X}_k \hat{X}_{i-k} - \hat{X}_i \sum_{j=1}^{n-i}(1 + \delta_{ij})\hat{X}_j = -\hat{X}_i^2 - \hat{X}_i \sum_{j=i+1}^{n-i} \hat{X}_j = 0, \tag{6}$$

and hence $\hat{X}_i = 0$. However, if $i > [n/2]$, the left hand side of (6) vanishes for arbitrary $\hat{X}_i > 0$, and hence the concentration of i-mer can remain positive at equilibrium if $i > [n/2]$ (if $i > 4$ in the

case of apoptosome with $n = 7$). Thus we conclude that

$$
\begin{aligned}
\hat{X}_i &= 0, \ \text{for } i = 1, 2, \cdots, [n/2] \\
\hat{X}_i &> 0, \ \text{for } i = [n/2] + 1, [n/2] + 2, \cdots, n
\end{aligned} \tag{7}
$$

This result indicates that the concentrations of intermediate oligomers, composed of less than the half of n molecules are completely consumed in the cell at equilibrium, but a certain amount of the intermediate oligomers consisting of more than half of n molecules remain unconverted at a certain level at equilibrium. Thus the efficiency of Apaf-1 heptamer assembly cannot reach 100% if n is greater than 3. The question then arises under what parameters, λ, C_0, and n, the efficiency of signal transduction is maximized, or, what parameters minimize the concentration of garbage intermediate oligomers that fail to be converted to active final product.

We first examine how the conversion efficiency $X_n/(1/n) = nX_n$ changes by changing the parameters $C_0 = c_0/y_0$, the ratio of the initial concentrations of cytochrome c to inactive Apaf-1. The effect of changing the initial concentration of cytochrome c on the time course of intermediate and the final assembled oligomers are shown in Fig. 3. The equilibrium concentration of X_7 is plotted against the initial concentration of cytochrome c in Fig. 4. While the initial concentration of cytochrome c is smaller than that of inactive Apaf-1, the final concentration of complete product increases approximately linearly with the initial concentration of cytochrome c. The conversion ratio nX_n, the equilibrium concentration of complete assembly relative to the one n-th of that of the initial inactive monomers, attains its maximum when the initial concentration of cytochrome c is equal to that of Apaf-1. When the initial concentration of cytochrome c is further increased, the final concentration of complete product start decreasing. For the sufficiently large initial amount of cytochrome c, the final concentration of complete product can become less than 50% of its potential maximum amount.

4 Transient behavior and finite time optimization

The speed of the production of intermediate Apaf-1 oligomers would affect the final concentration of the complete assembly. If Apaf-1 oligomers are slowly supplied, so monomers are produced first, and then are dimers, trimers and so on, every intermediate oligomers have a higher chance of contributing to the formation a complete assembly, than when all sizes of oligomers are produced at once. Because the rate of changes of cytochrome c and inactive Apaf-1 are the same, the difference between cytochrome c and inactive Apaf-1 is kept constant. If $c_0 > y_0$, therefore,

$$\frac{dy}{dt} = -\alpha(c_0 - y_0 + y)y$$

and

$$y(t) \quad = \quad \frac{y_0(c_0 - y_0)}{c_0} \frac{1}{e^{\alpha(c_0 - y_0)t} - \frac{y_0}{c_0}} \sim \text{const.} \times e^{-\alpha(c_0 - y_0)t} \tag{8}$$

If $c_0 = y_0$, on the other hand

$$\frac{dy}{dt} = -\alpha y^2$$

and

$$y(t) = \frac{1}{\frac{1}{y_0} + \alpha t} \sim \text{const.} \times t^{-1} \tag{9}$$

Thus, if the initial concentration of cytochrome c is the same or close to that of inactive Apaf-1, the inactive Apaf-1 decreases only geometrically slowly. However, if they are different, the inactive Apaf-1 decreases exponentially fast (Fig. 5).

We denote by T_{50} the time until the concentration of the complete Apaf-1 heptamer reaches 50% of the final concentration. The parameter dependence of T_{50} is summarized in Fig. 6. In the case of $n = 7$, T_{50} monotonically decreases with c_0/y_0 in almost all range of c_0/y_0 except when it is very close to 1. The speed of the Apaf-1 activation is thus accelerated by increasing the amount of cytochrome c release. When $c_0 = y_0$, however, Apaf-1 is slowly activated as described previously, giving rise to a local peak of T_{50} around $c_0 = y_0$.

Though the conversion efficiency at equilibrium is maximized at $c_0/y_0 = 1$, the time required for the accumulation of the final product becomes longer as c_0/y_0 approaches 1 (Fig. 6.) To examine the conversion efficiency in biologically plausible time scale, we therefore consider the concentration of complete Apaf-1 heptamer after a certain fixed time has passed. As shown in Fig 7, the concentration of complete Apaf-1 heptamer at a prefixed time becomes high when c_0/y_0 is large and α/β is small. There is a certain optimal c_0/y_0 for a given fixed α/β. Thus, if α/β is sufficiently small, the optimal c_0/y_0 ratio can be far larger than 1. This is quite different from the conversion ratio at *equilibrium* (Fig 8). The area for high concentration of complete Apaf-1 heptamer is restricted in the region near $c_0/y_0 = 1.0$.

5 Tetramer is the most efficient in conversion ratio

At equilibrium after sufficiently long time has passed, the concentration of complete Apaf-1 heptamer for fixed α/β is maximized when $c_0/y_0 = 1.0$, because Apaf-1 activation proceed geometrically slowly when $c_0/y_0 = 1.0$ as mentioned previously. The equilibrium concentration of the complete assembly changes also with the number of molecules n that comprises the final assembly, as shown in Fig. 4. This result shows that the tetramer ($n = 4$) is the most efficient complex for the conversion independent of the initial concentration of cytochrome c. The conversion ratio does not related to biological function. The reason why the actual apoptosome is composed of heptamer cannot be explained by the conversion ratio. It is necessary to consider other reasons why heptamer is adopted with the actual apoptosome. If the conversion ratio of signal to final product is to be maximized, the tetramer is the most efficient among oligomers more than two units. In this respect, the fact that Apaf-1 heptamer, rather than tetramer, is used is not understood only from the optimization of the conversion ratio. The reason why organism uses heptamers might therefore be explained by other hypothesis such as improved tolerance to noise by virtue of a greater nonlinearity of response to signal by using oligomers.

6 Effect of dissociation

Here we consider the dissociation of Apaf-1 oligomers by restoring a positive γ in (3). Though Apaf-1 heptamer can be dissociated into smaller oligomers, an introduction of dissociation increases the final concentration of Apaf-1 heptamer. This is because once produced intermediate oligomers can be reconverted into smaller oligomers that contribute to further production of heptamers. Fig 9 illustrate this point. The equilibrium concentration is maximized at an intermediate γ, because high concentration of heptamer cannot be maintained with too large dissociation rate.

As shown in Fig 9, when the dissociation constant is sufficiently small, the concentration of complete Apaf-1 heptamer reaches a plateau at a low level that corresponds to the equilibrium concentration of (4) that ignores dissociation (compare the trajectories for $\gamma = 0$ and $\gamma = 10^{-4}$ in Fig. 9). More specifically, this is expected if $1/\gamma$ is sufficiently smaller than the T_{50} for $\gamma = 0$, the time until the concentration of the final product reaches half of its equilibrium level.

Therefore, if $1/\gamma$ is much longer than biologically interesting time scale, the results we obtained in the previous sections by ignoring dissociation process can well approximate the behavior of full kinetics.

7 Discussion

We construct a simple mathematical model for the Apaf-1 heptamer assembly process. We have analytically shown, as in Eq. (7) in the text, that the substantial amount of intermediate oligomers can remain at quasi-equilibrium before the dissociation of oligomers begins. The intermediate oligomers composed of 6, instead of 7, Apaf-1 molecules are indeed found in *in vitro* assembly of apoptosome(Acehan *et al.*, 2002). Our model predicts that tetramers, pentamers, and hexamers remain at equilibrium, and that among them hexamers are the most abundant (see Fig. 3). Though we focussed on the Apaf-1 heptamer assembly with $n = 7$ unit proteins, our model can be applied

to the assembly process with arbitrary number of unit proteins. Our model then reveals that a large protein complex composed of, and assembled from, greater than 3 unit proteins is disadvantageous in terms of the conversion efficiency in the signal transduction, because the amount of unconverted intermediate oligomers increases with the number of unit proteins n if $n > 3$. As shown in Fig. 4, if the others are the same, $n = 4$ (tetramer) is the optimum number of unit molecules that compose of complete assembly, which minimizes the amount of unconverted intermediate proteins. The conversion efficiency Apaf-1 heptamer formation (nX_n) also changes with the other parameters than n. Our non-dimensionalized model (5) reveals that the concentration of each oligomer depends on the two parameters, the concentration of released cytochrome c relative to the initial inactive Apaf-1 concentration (c_0/y_0), and the ratio of the association rate of inactive Apaf-1 and cytochrome c relative to the association rate of oligomerization of active Apaf-1 oligomers (α/β), in the following ways.

If we focus on the equilibrium conversion ratio of Apaf-1 monomers to Apaf-1 heptamers, we see from Fig. 8 that the region for high conversion efficiency is restricted in the region near $c_0/y_0 = 1.0$. The conversion efficiency in *in vitro* Apaf-1 heptamer assembly experiment is found to be close to 1 (Acehan *et al.*, 2002), and, in the experiment, the molar ratio of Apaf-1 to cytochrome c was indeed adjusted from 1:1 to 1:2.

Once triggered, all cytochrome c's stored in mitochondria are released, regardless of the duration and the strength of stimulus of the apoptotic signal (Goldstein *et al.*, 2000). The ratio c_0/y_0 in our model might therefore be much larger than 1 in the cells that undergo apoptosis through mitochondrial pathway. We have shown in Fig 7 that the concentration of Apaf-1 heptamers that is produced within a finite time since the cell received the signal is maximized at c_0/y_0 larger than 1 when α/β, the ratio of the activation rate of monomers to the association constant for oligomerization, is small. Though slow supply of the active Apaf-1 monomers increases the chance for the intermediate oligomers to contribute to the formation of a complete assembly, it also slows down the whole process of assembly production. This makes an intermediate speed of Apaf-1

activation optimum for efficient production of Apaf-1 heptamers in a finite time. From equations (4), we see that the speed of Apaf-1 activation is proportional to αc. Therefore the region for high concentration of Apaf-1 heptamers exists along the line $\alpha c_0 = \text{constant}$ in the parameter space (see Fig 7). The activation rate of Apaf-1 monomer, α, must be decreased as the amount of cytochrome c increases to keep the speed of Apaf-1 activation at an intermediate optimum level.

As is discussed above, it is necessary to reduce the speed of Apaf-1 activation under excessive cytochrome c release to maximizes the accumulation speed of Apaf-1 heptamers in a finite time. The mechanism regulating the speed of Apaf-1 activation exists in cells. It is reported that the interaction between cytochrome c and Apaf-1 depends on the ionic strength(Purring *et al.*, 1999). Cytochrome c and Apaf-1 are positively and negatively charged, respectively. The *in vitro* experiment has shown that the concentration of cation in the reaction buffer inhibits the interaction between cytochrome c and Apaf-1 (Purring-Koch & McLendon, 2000). The alteration of the concentration of cytoplasmic cation by calcium flux may regulate the association rate α of interaction between cytochrome c and Apaf-1. Inhibition of interaction between cytochrome c and Apaf-1 by calcium may decelerate the speed of Apaf-1 activation.

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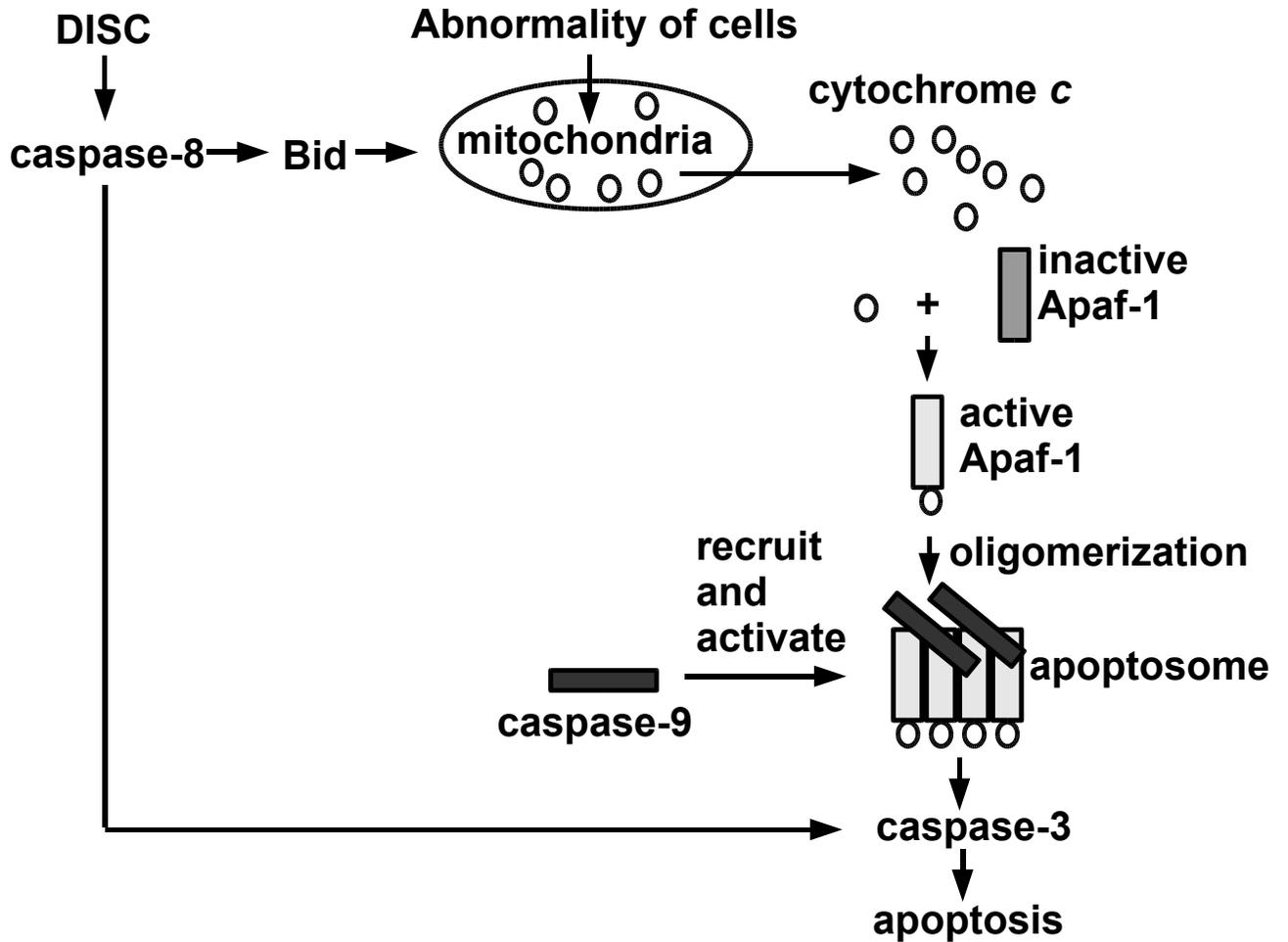
Figure 1: Schematic view of the extrinsic and the intrinsic triggering pathway of apoptotic signal cascade. Either the activation of the caspase-8 and subsequent activation of Bid, or abnormality of cells, triggers the release of cytochrome c from mitochondria to cytoplasm. Cytoplasmic cytochrome c binds the Apaf-1 protein. This interaction introduces the conformational change of the Apaf-1, leading to an activated Apaf-1. Activated Apaf-1 monomer or oligomer can interact with each other and form a larger oligomer, and this assembly process finally ends up with the production of heptamer protein complex called apoptosome. Caspase-9 is activated in apoptosome without cleavage. Activated caspase-9 can cleave and activate the executioner caspase-3. Active complex of caspase-3 then causes the apoptosis.
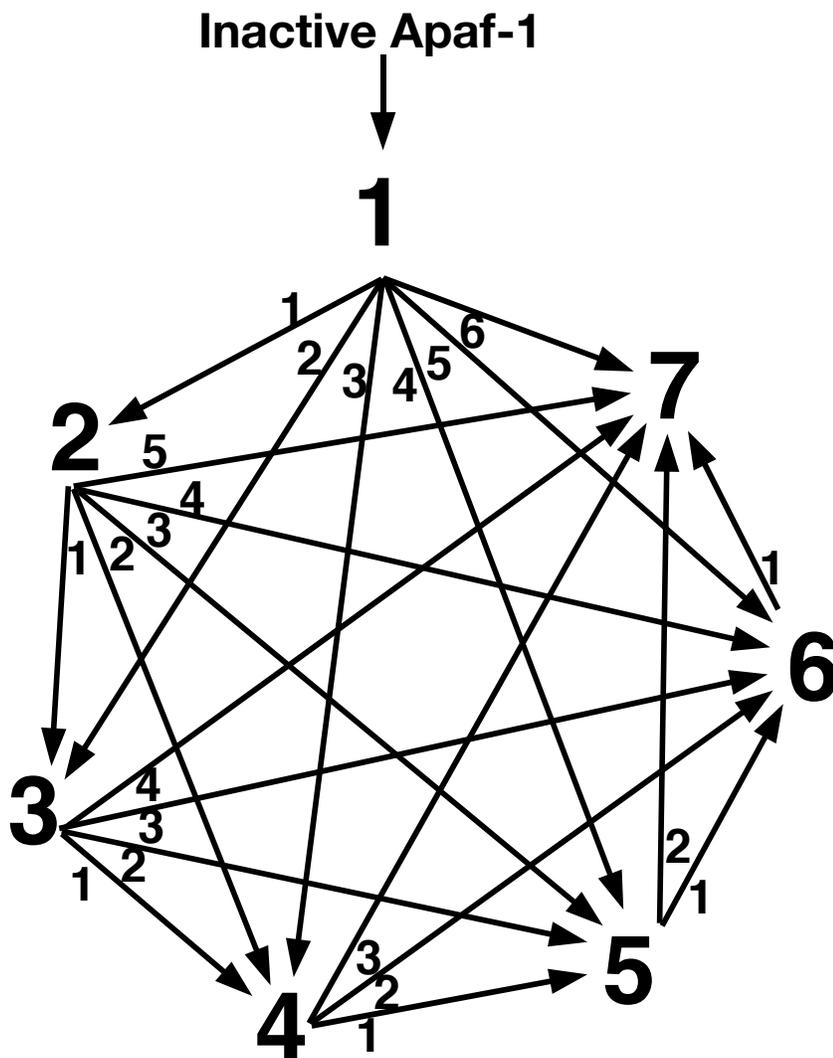
Figure 2: The diagram of oligomerization of Apaf-1. The oligomer composed of i molecules of Apaf-1 can interact with oligomer composed of j molecules of Apaf-1 only when $i + j$ is less than 7, which results in a larger oligomer composed of $i + j$ molecules of Apaf-1.
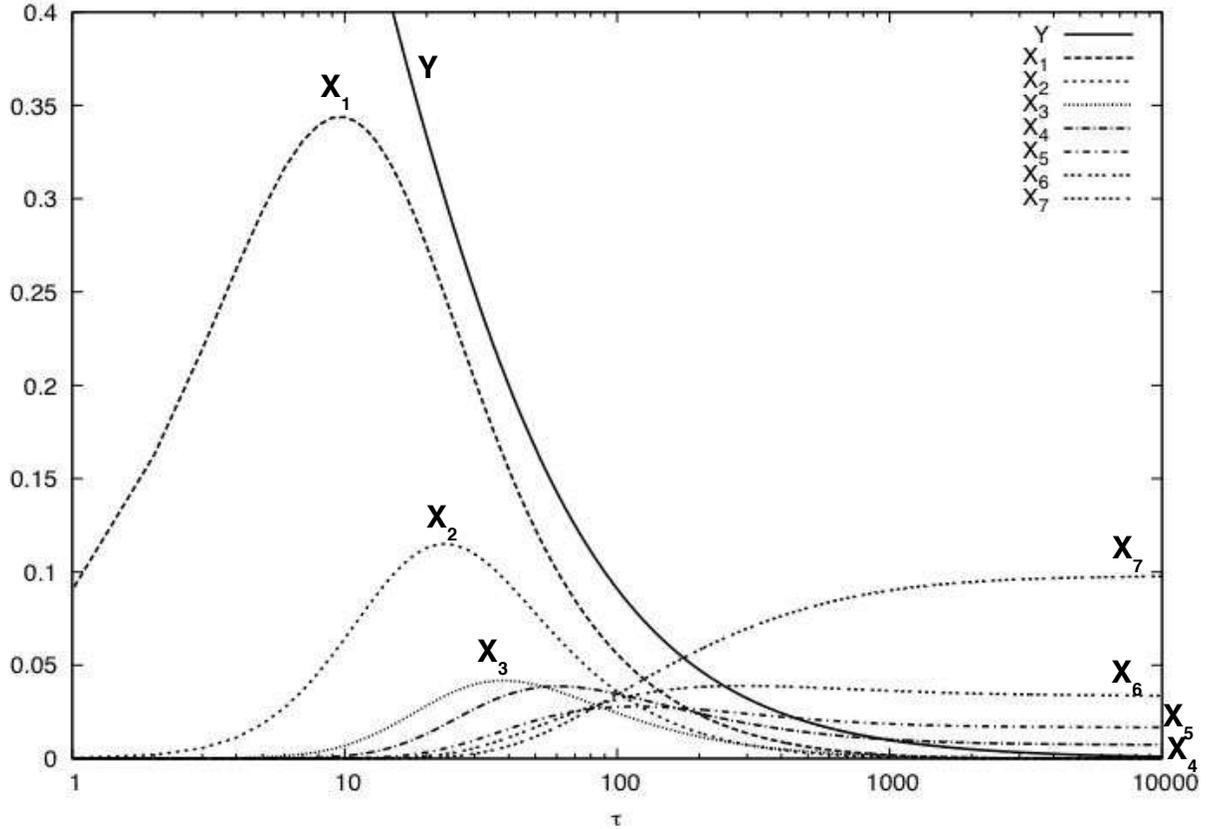
Figure 3: The time course of the concentrations of the each oligomers. The apoptotic signal is added to the cell at time $\tau = 0$, and cytochrome c of the amount c_0 is released in cytoplasm at the same time. Inactive Apaf-1 designated by Y is consumed by the interaction with cytochrome c and its concentration decreases. The concentration of intermediate Apaf-1 oligomers increase step by step. Monomers, dimers and trimers are all consumed for the production of larger oligomers after they increased once. The concentrations of tetramers and larger oligomers also attain their peaks one by one, and start declining thereafter. However, their concentrations remains positive when all reactions are stopped. Parameters: $n = 7$, $c_0 = 70.0[\mu M]$, $y_0 = 70.0[\mu M]$, $\alpha = \beta = 0.004[\mu M^{-1}s^{-1}]$. The trajectories are obtained from equations (5) with the rescaled parameter values $C_0 = c_0/y_0 = 1.0$ and $\lambda = \alpha/\beta = 1.0$. The time axis is scaled logarithmically.
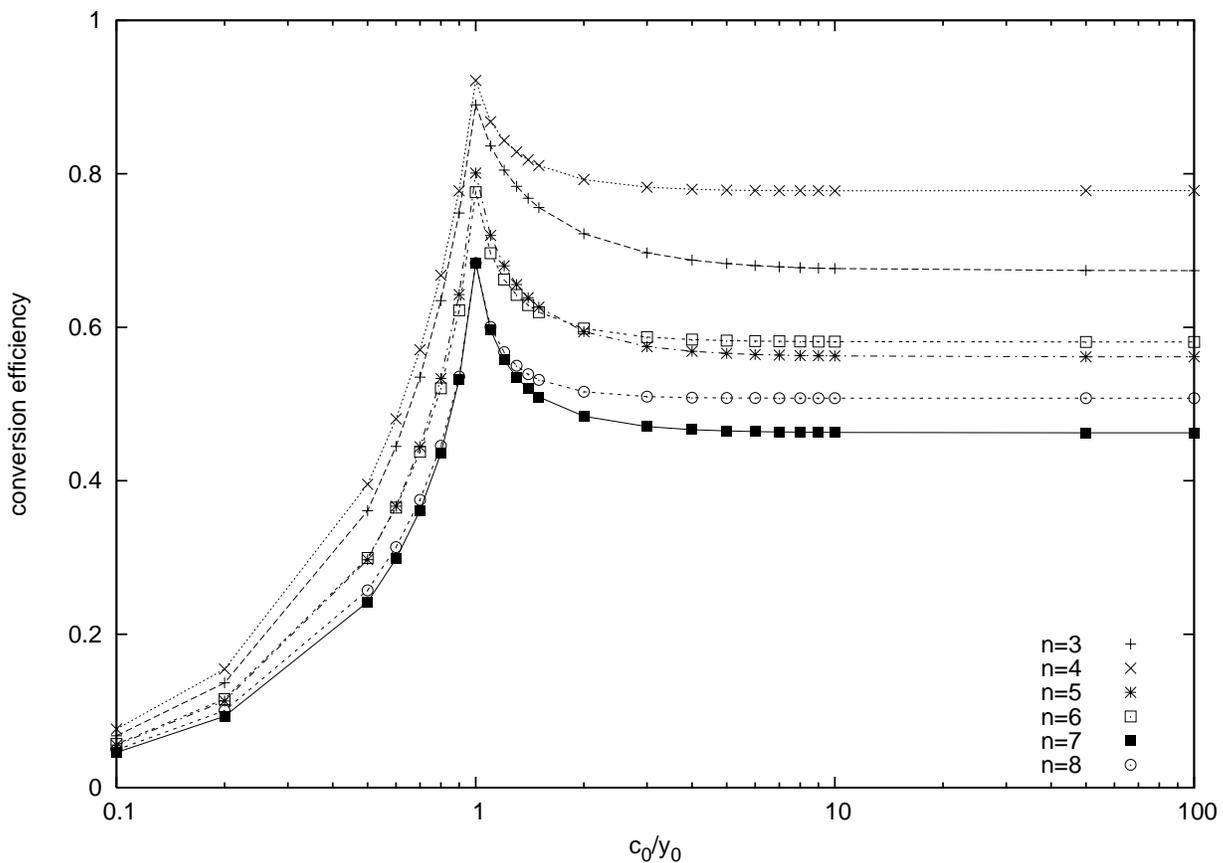
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Figure 4: The final concentration of Apaf-1 heptamer relative to the one n-th of the initial concentration of inactive Apaf-1 (nX_n), which represents the conversion efficiency of Apaf-1 heptamer assembly process. The horizontal axis indicates the ratio of the initial concentration of cytochrome c to that of Apaf-1. The vertical axis indicates the final concentration of the complete products composed of different number n of unit molecules.
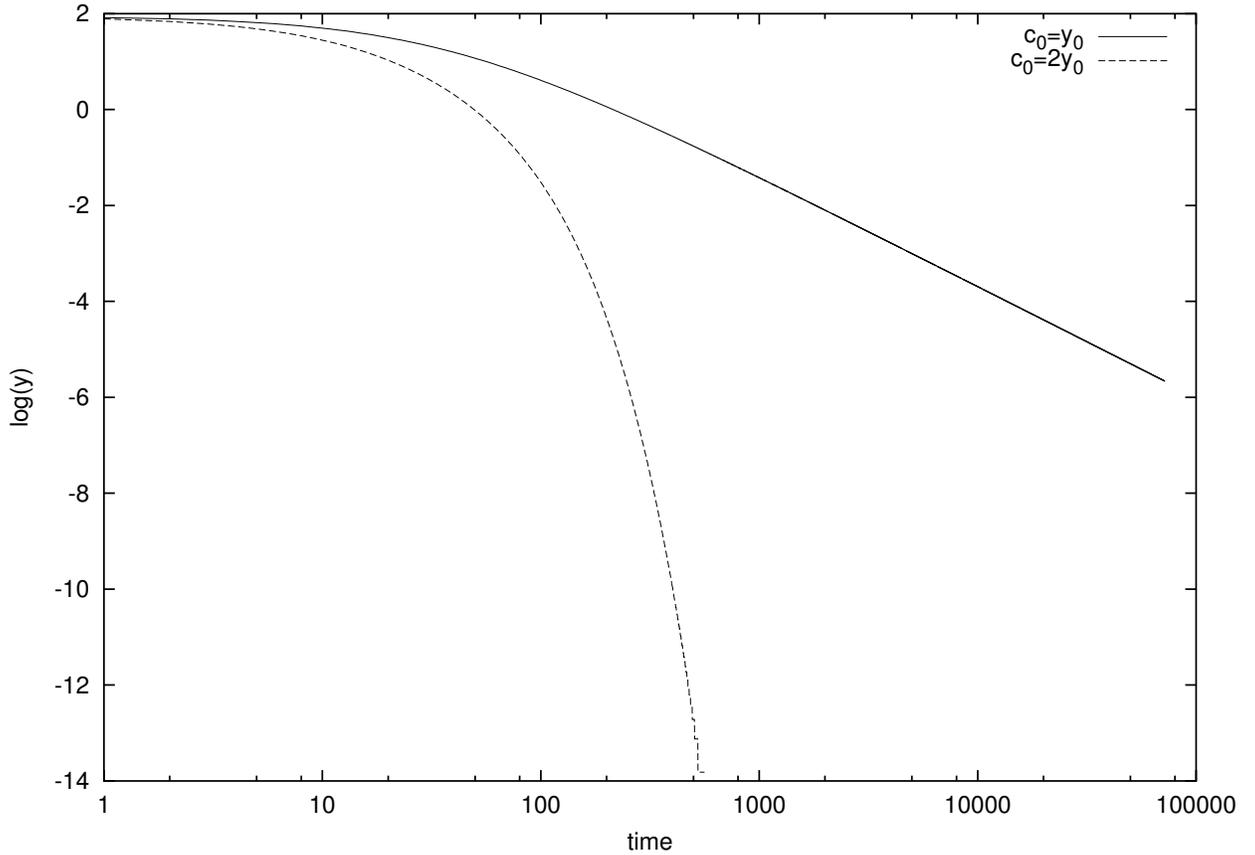
Figure 5: The time course of the consumption of inactive Apaf-1. Inactive Apaf-1 is consumed by its binding to cytochrome c. The speed of the consumption of inactive Apaf-1 depends on the ratio of the initial concentration of cytochrome c to that of inactive Apaf-1. The speed of the decline of inactive Apaf-1 strongly affects the final concentration of complete product, because all reactions have to stop when the concentration of small oligomers composed of less than half of n molecules becomes 0. Dotted line show the case if $c_0 > y_0$ (in this plot $c_0 = 2 \times y_0$), inactive Apaf-1 decreases exponentially. Whereas, solid line show the case if $c_0 = y_0$, the concentration of inactive Apaf-1 decreases only geometrically slowly – $y(t)$ is proportional to the reciprocal of time.
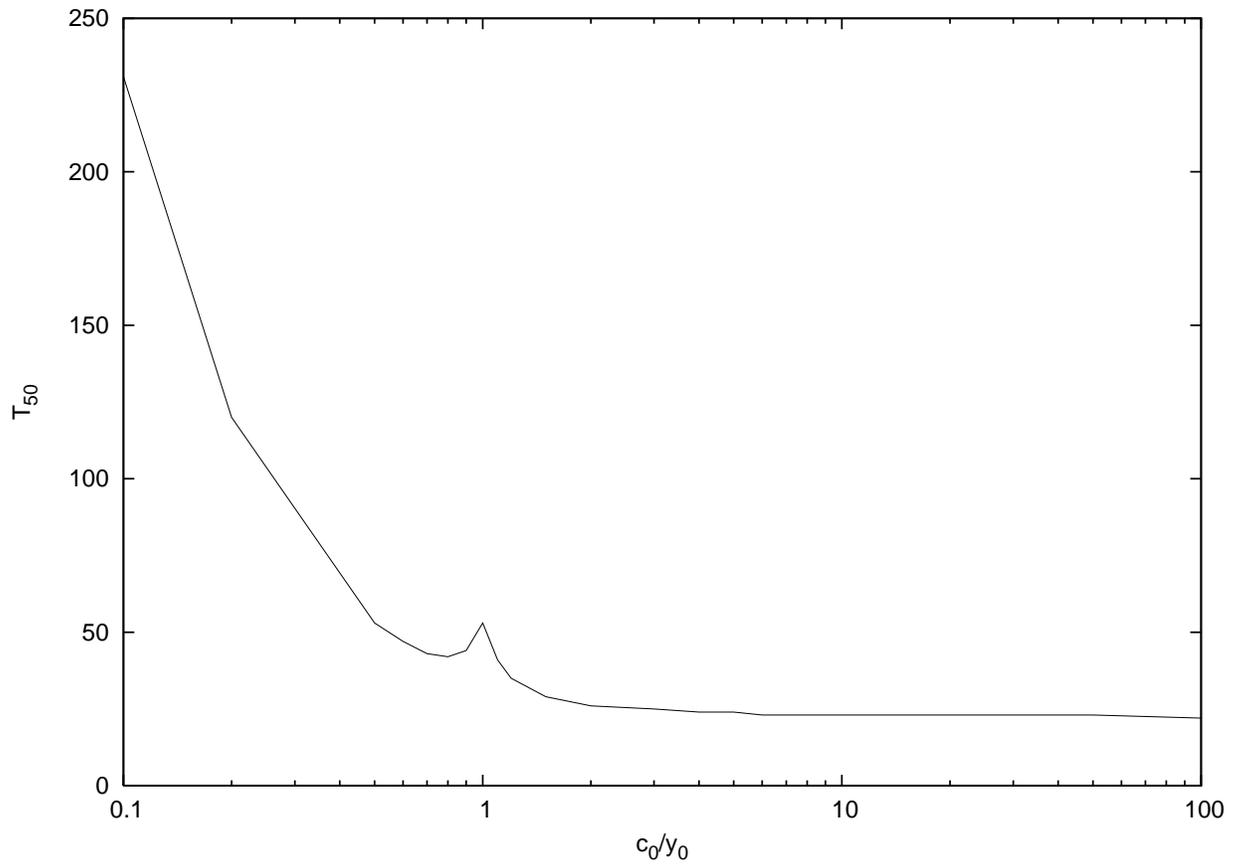
Figure 6: The speed of the apoptotic signal transduction, measured with T_{50}, the time until the concentration of complete product reaches the half of its final concentration. The reciprocal of T_{50} represents the speed of the apoptotic signal transduction. T_{50} decreases with c_0/y_0 in almost all its range except when $c_0 \approx y_0$. When $c_0 = y_0$, the reaction proceeds geometrically slowly, and hence T_{50} has a small local peak around it.
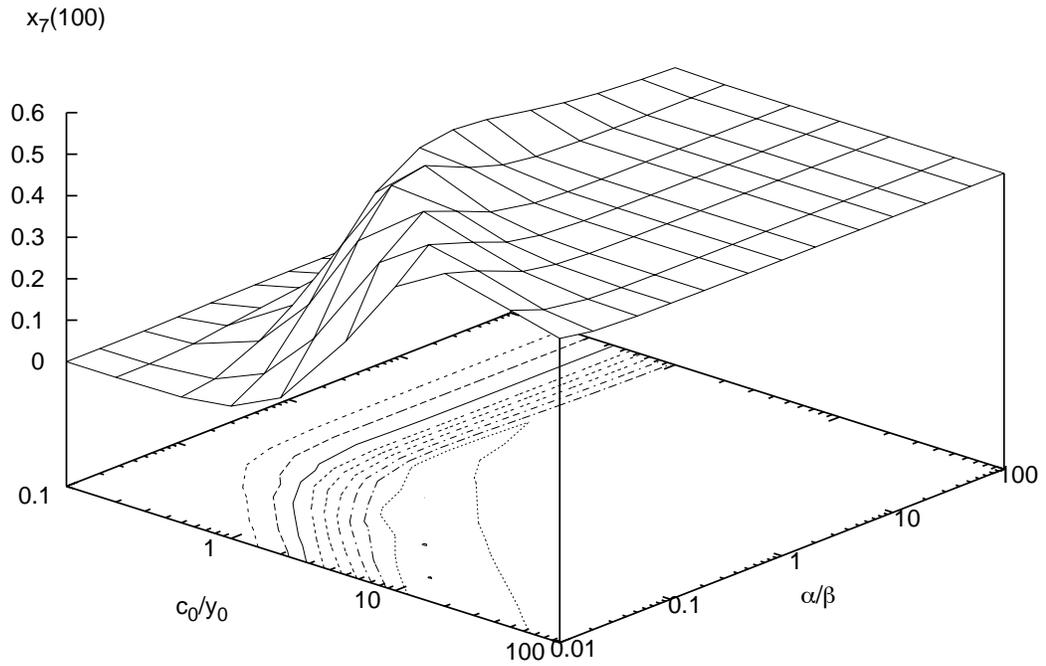
Figure 7: The contour plot for the concentration of complete Apaf-1 heptamer, $x_7(t)$, at time $t = 100.0[s]$ after the release of cytochrome c, calculated from equations (4). For a fixed α/β, there is an optimum c_0/y_0, indicating that neither too few nor too much release of cytochrome c is advantageous for the efficiency of complete Apaf-1 heptamer assembly. Likewise, for a fixed c_0/y_0, there is an optimum α/β ratio.
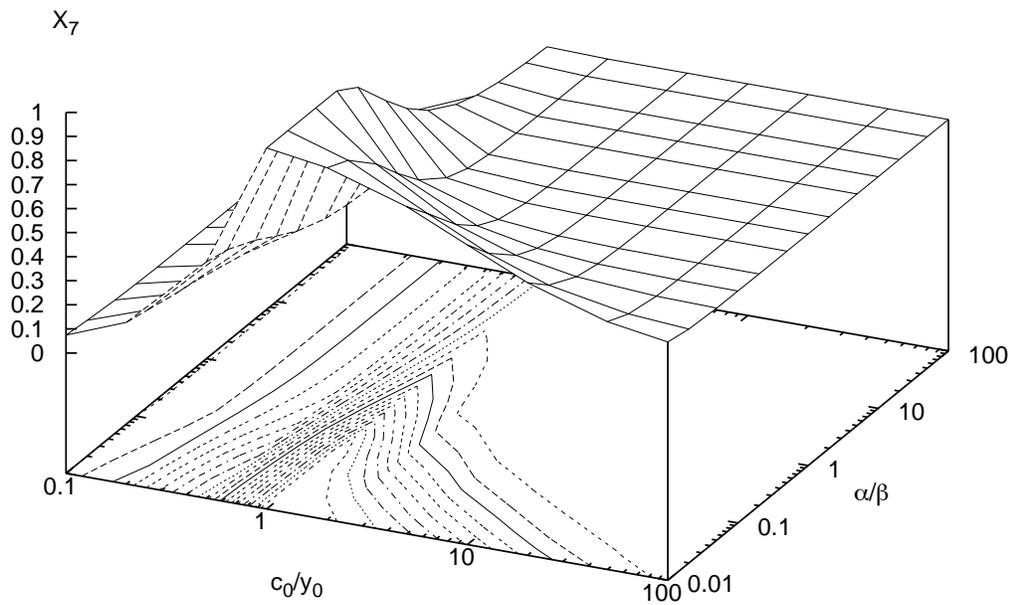
Figure 8: The contour plot for the final concentration $X_7(\infty)$ of Apaf-1 heptamer in the apoptotic signal transduction, plotting against α/β and c_0/y_0, calculated from equations (5). In contrast to the results for a finite time case (Fig 7), the area for high concentration of complete Apaf-1 heptamer is restricted in the region near $c_0/y_0 = 1.0$. The final concentration monotonically increases with decreasing α/β. A small α/β implies a slow production of active Apaf-1 monomers, which increases the conversion efficiency of the Apaf-1 heptamer assembly.
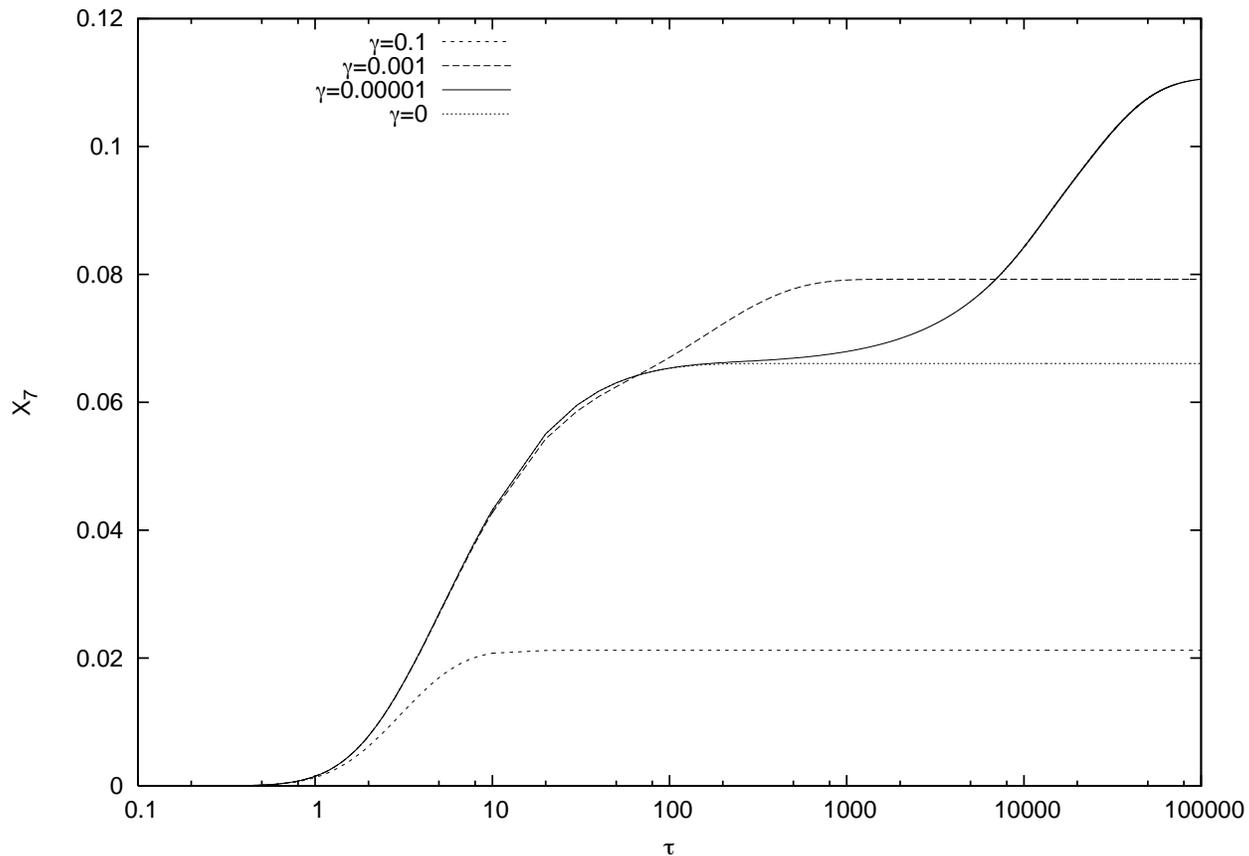
Figure 9: The time changes of the concentration of complete Apaf-1 heptamer for various γ, with $c_0 \gg y_0$. When γ is small, the mean relaxation time for the dissociation of intermediate oligomers is very long, and the complete Apaf-1 heptamer reaches a plateau before substantial dissociation occurs. The results with nonzero dissociation fail to show any significant difference from the result without dissociation when γ is sufficiently small. Therefore, for sufficiently small γ, the approximation that neglects the dissociation of intermediate oligomers can be guaranteed. Parameters: $\alpha = \beta = 0.004[\mu\mathrm{M}^{-1}s^{-1}]$.