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Steady state approximation in the minimal model of the alternative pathway of complement

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Abstract

Complement is a response mechanism of the immune system. Two initiation pathways have been characterized for complement. The classical pathway is antibody mediated while the alternative pathway is not. Since the alternative pathway is independent of antibodies, it is always active. For the alternative pathway we have previously developed a minimal model. Using parameters within physiological bounds, the model showed complex behavior also within physiological bounds. Thus the model seems to be an appropriate representation of the alternative pathway response. By applying a steady state assumption to the Michaelis Menten step of the minimal model, we reduce the number of variables from six to five. A comparison between the dynamics of the minimal and contracted models reveals that the two descriptions may not be compatible. Although both systems show chaotic behavior it occurs in different regions of parameter space. © 1997 Elsevier Science B.V.

Keywords: Alternative initiation pathway; Complement; Minimal model; Steady state approximation

1. Introduction

The skin, ciliated mucous openings, gastric juices, and various intestinal bacteria make up the immune system's first line of defense. These are all nondiscriminating barriers to infection. Once a microbe penetrates any of these boundaries, the immune system responds [1–4]. It is essential that the response is able to distinguish between self and non-self. Two response modes exist, these are cell and humoral mediated [5]. For the cell mediated response, various macromolecules (usually proteins or polysaccharides) on the cell membrane play a key role in identification. Self cells are marked by a class of proteins on their membrane called the major histocompatibility complex (MHC) markers, while the surface of foreign agents contains antigens, macromolecules capable of triggering an immune response. Cell mediated response is carried out by T cells. The T cells release perforin and other chemicals which cause cell lysis upon interaction with both MHC and antigen markers on the cell surface. This response targets virus-infected cells. The humoral mediated response is carried out by macrophages, B cells, and complement; it is responsible for destroying bacteria, viruses in extra cellular phase, fungi, and protozoans. When macrophages recognize an alien substance, they partially engulf it, keeping the antigenic portion

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intact. This stimulates the growth of B cells, some of which transform into plasma cells which produce antibodies to bind the antigen on the invader and tag it for destruction by complement or macrophages.

Complement is composed of plasma proteins and membrane proteins which function as enzymes or binding proteins to cause cell lysis [3,4]. Several activation pathways for complement have been characterized; the two best known are classical and alternative. Unlike all other pathways, the classical pathway is triggered by the presence of antibodies, while the others, including the alternative, are antibody independent. Because the classical pathway is antibody-mediated, its activation is more specific to foreign species, while the antibody independent pathways attack self and non-self cells indiscriminately. Antibody independent responses are critical in the early stages of infection before antibodies have been synthesized, a process which takes several days.

The alternative pathway could be triggered by simple molecular structures such as polysaccharides, lipopolysaccharides, and teichoic acid, which have repetitive units and are present on the surfaces of bacteria, fungi, viruses, parasites, tumor cells, certain mammalian cells and aggregates of immunoglobulins. Denatured DNA, proteins released from cell death, and injured tissue can also activate the alternative pathway.

The classical and the alternative pathways converge in the formation of the membrane attack complex (MAC). The MAC embeds itself in the membrane of the target cell, a foreign cell or infected host cell, through a hydrophobic interaction with the lipid bilayer. It then unfolds and polymerizes. This process debilitates the target cell's membrane structure, creating transmembrane channels through which the cell's plasma flows and eventually causing cytolysis of the cell.

The use of differential equations to model complex biochemical systems plays an increasing role in the prediction and interpretation of familiar biological phenomena. These models often contract multistep processes into one step. The drawback of such simplifications is that in some cases the results may not reflect the actual behavior of the system. However, if such simplifications are performed carefully, the resulting model is a minimal one devoid of irrelevant mechanistic details. The temporal behavior of various components of the minimal model can then be studied in order to explore the effects of initial conditions and parameter values. Understanding these effects will help us determine the rules governing the temporal behavior of the system.

To date, biochemistry has focused upon isolated and closed systems which function near equilibrium. The introduction of mathematical models enables the study of open systems far from equilibrium. Such systems can have multiple steady states, as well as spatial and temporal oscillatory states. Previous use of differential equations to model systems has been hampered by the fact that there are only a reduced number of differential equations that can be solved in closed form. These include first order, exact, linear, and homogeneous equations, higher order linear equations with constant coefficients, and partial differential equations which are reducible to these equations by the separation of variables. Approximate solutions to differential equations outside these categories can be attempted. For example, it is possible sometimes to use analytical approaches, such as perturbation theory [6], bifurcation theory [7], and differential topology [8,9], in the analysis of dynamical systems. Also, solutions to the system of ordinary differential equations (ODEs) by numerically integrating the equations using appropriate algorithms [10] is a common tool. The advantage of numerical analysis is that it is possible to carry out integrations over a whole host of parameters, whereas analytical methods are limited to parameter values that are close to solvable solutions.

Modeling involves more than deriving a set of equations to represent the system. Part of the challenge is to find parameters. As it turns out, many models for complex biological systems have been found to be chaotic for some choice of the parameters due to nonlinearities in biochemical reactions [11–16]. The deterministic chaos in some cases is constrained to well-defined intervals of metabolite concentrations.

Chaotic mechanisms may be related to a response mechanism (or pathology) whereby switching from an oscillatory to chaotic scheme compensates (or overcompensates) for a given stimulus [12]. Furthermore, chaotic schemes may be responsible for the biological diversity necessary for species to survive. There is a strong sense that the understanding of

temporal patterns will provide a means of understanding natural phenomena.

Previous studies in chemical and biochemical systems have revealed chaotic and periodic oscillations [13–16]. Some examples of oscillating reactions are biological clock functions, [17] population growth [18], and positive and negative feedback loops such as the peroxidase reaction and glycolysis [11]. These are all rough scenarios where the gradual accumulation of a metabolite occurs to a maximum level after which a gradual decline in the metabolite concentration occurs to some lower bound where the cycle continues on upwards again. Also in the case of the peroxidase reaction and of glycolysis, chaotic oscillations have been reported. [14] Recently, we have reported chaotic oscillations in our minimal model of the alternative pathway of the complement [19], where we have used physiological parameters.

In Section 2, we review the Complement system. In Section 3, we discuss the minimal model of the alternative pathway. In Section 4, we contract the minimal model using steady state approximations to a five variable model. Numerical analysis of both the minimal and the contracted model are then compared. Finally, we discuss our result in Section 5.

2. Complement system

The proteins of the pathways of complement are termed components, and labeled C1 through C9. They are all distinct plasma glycoproteins, except for C1 which fragments into three glycoproteins, C1r, C1q, and C1s. Multiple polypeptide chains which form inactivated components are distinguished by Greek suffixes with α denoting the longest chain, then β, and so on. Cleavage fragments are indicated by a suffixed lower case letter. The enzyme activated form of a component is indicated by a bar over its name. Components C1–C4 are involved in the classical pathway. Regulators of the classical pathway are abbreviated according to their function, e.g., C1 inhibitor is C1inh. The rest of the proteins, components C5–C9, make up the MAC. In addition C3 is involved in the alternative pathway along with other proteins called factors. There are three factors involved in the initiation and amplification of the alternative pathway: B, D, and P (properdin). In addition, factors H and I regulate the response of the alternative pathway.

The complement response is an enzyme cascade. An enzyme cascade is defined as a series of reactions in which the products catalyze the next step in the series. Enzyme cascades probably evolved because they are critical for quick reactions to stimuli. The most commonly known example of a cascade is the fibrinolytic cascade which controls the formation of blood clots. It is important that cascades are regulated. On the one hand, the appearance and size of blood clots must be monitored, otherwise clots could potentially grow to a size that would block circulation. On the other hand, the absence or slow formation of a blood clot, when necessary, would result in excessive blood loss.

There are two general types of enzyme cascades, open and damped. Open cascades have stable products which catalyze the next step in the cascade. In contrast the products of damped cascades are unstable and must react with the substrate of the following reaction quickly, otherwise it is broken down. Since the intermediates formed during a complement response are metastable and subject to regulation by various methods, complement can be regarded as a damped cascade with a complex regulatory mechanism.

2.1. The alternative pathway

Six proteins initiate, recognize, and amplify the alternative pathway, C3, B, D, H, I, and P. The only component involved in the alternative pathway is C3, and it is biologically inactive since C3 reveals no binding site with which cells can interact. The activation of C3 is accomplished by a spontaneous hydrolization. The spontaneous hydrolysis of the thiolester of C3 yields $C3(H_2O)$. The hydrolyzed form, stable for less than a second, can bind factor B in the presence of Mg^{2+} to form $C3(H_2O)$,B. Cleavage of $C3(H_2O)$B by factor D yields inactive Ba and active initial C3 convertase, $C3(H_2O)$,Bb. This initial C3 convertase can then go on to cleave C3 into C3a and C3b. Compared to the thiolester in C3 ($t_{1/2} = 231$ hr at 37°C), the thiolester in C3b is 10^{10} times more reactive ($t_{1/2} = 60$ μs) [20]. The thiolester on C3b can bind nucleophiles such as hydroxyl and amino groups found on the surfaces of cells. The surface

bound C3b can then bind factor B in the presence of Mg^{2+} to form C3b,B which is subsequently cleaved by factor D to form surface bound C3 convertase, C3b,Bb. Instead of attaching to a surface, the active thiolester of C3b can go through the subsequent steps and result in the formation of fluid phase C3 convertase, C3b, Bb. Both forms of C3 convertase continue to cleave C3 and thus amplify the amount of C3 convertase. Finally the accumulation of surface bound C3 convertase enables the formation of the MAC.

Typical of enzyme cascade systems, steps of regulation exist along the pathway. Several regulatory plasma and membrane proteins exist which mediate the autocatalysis of C3 convertase. Such regulation is necessary in order that the accumulation of C3 convertase does not occur on the surfaces of the self and nonself cells indiscriminately. C3 convertase is unstable with a half-life of 90 sec at 30°C and an enzyme specific activity, k_{cat}/K_m, between 1.6×10^5 and 3.1×10^5 M^{-1} s^{-1} [20]. The binding of unstable C3 convertase to P to form C3b,Bb,P stabilizes the enzyme. Factor H, decay accelerating factor (DAF), or complement receptor type 1 (CR1) can bind C3 convertase and P stabilized C3 convertase to catalyze the decay of C3 convertase to C3b and Bb. Proteolytic inactivation of C3b can also be catalyzed by factor I in the presence of cofactor H, membrane cofactor protein (MCP), or CR1. Membrane proteins MCP and DAF have a wide tissue distribution, while CR1 is a membrane protein with a more limited tissue distribution. Together these are the protective surface proteins located on host cells. Acceleration of the decay of initial C3 convertase is carried out by factor I in the presence of cofactor H on the $C3(H_2O)$ portion of initial C3 convertase. The preference for factor B is slightly more than that for factor H and thus $C3(H_2O)$ has a slightly greater chance of forming initial C3 convertase than being degraded by factors H and I. Fraction C3b normally binds factor H preferentially, but in the presence of an infection the affinity of C3b for factor B increases substantially. In addition, factor H binds C3b on host cells 100 times more than on invading cells; on invading cells factor H and B show similar binding preferences. Factor H cannot readily access C3b bound on activating surfaces.

The actual structures which activate the alternative pathway are not known, but the trigger necessarily involves the recognition by C3b. The environment which surrounds the surface onto which C3b is bound is also critical to the response since a preference for factor B will amplify the response whereas a preference for factor H will terminate the response.

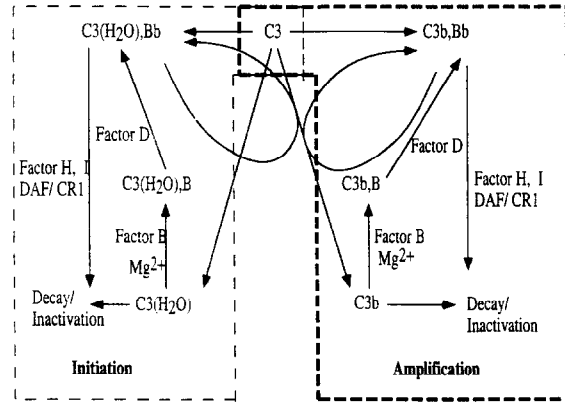

Fig. 1. Schematic of the Alternative Pathway of Complement.

The formation of a second C3b molecule by C3 convertase can couple with a surface bound enzyme in the presence of P to form C3bPC3b which is the C5 convertase of the alternative pathway. Once C5 is cleaved by C5 convertase, complement is no longer reversible. The cleavage of C5 sets off a cascade which involves C5–C9 and terminates in the formation of the MAC. Though the formation of MAC is irreversible, there are membrane proteins, homologous restriction factor (HRF) and CD59, which prevent MAC insertion into host cells, thus preventing cell destruction. A summary of the alternative pathway is given in Fig. 1.

3. Minimal model of the alternative pathway

Previous analyses of the kinetics of complement involved approximations which preclude an examination of the kinetic behavior of the individual components [20]. The challenge to studying the kinetics of complement in vitro is that one cannot simply measure C3 to see if complement activation is occuring because inability to separate complement from other proinflammatory systems makes it difficult to

study. Thus this makes complement an ideal candidate for mathematical modeling.

A minimal model of the alternative pathway of complement has been proposed and its dynamics examined [21,22]. This model is unique in that it was designed to be mechanistically accurate. Previous models were designed following mathematical dictates after which a mechanism was proposed to fit the observed dynamics. This model, since it is based on the dictates of the complement system, is expected to be a better representation of the dynamics of the alternative pathway of complement. For this model, numerical integration has been used to characterize its dynamics.

The minimal model of the alternative pathway begins by modeling the spontaneous hydrolysis of C3. This step is assumed to be first order since water is present in excess in the body,

$$A \xrightarrow{k_1} B2 \tag{1}$$

where A represents C3 and B2 is $C3(H_2O)$. The concentration of C3 is regulated by the liver which responds to the excess or lack of C3 accordingly. Eq. (1) can be altered slightly to reflect the influence of the liver as

$$\text{liver}(A_0 - A) \xrightarrow{k_0} A \xrightarrow{k_1} B2 \tag{2}$$

The hydrolyzed C3 can be inhibited. This is represented as

$$B2 \xrightarrow{k_{d_1}} I \tag{3}$$

where I is the inhibited form of the enzyme. The hydrolyzed C3 can also, in a two step reaction with factors B and D and Mg^{2+}, form initial C3 convertase, $C3(H_2O)$,Bb. This process can be represented as

$$C3(H_2O) + B \rightleftarrows C3(H_2O),Bb \tag{4}$$

The rate determining step in Eq. (4) is the binding of factor B to the hydrolyzed C3. Once this occurs the uptake of factor D occurs readily. Thus Eq. (4) can be contracted into the following one step:

$$B2 \underset{k_{a_1}^-}{\overset{k_{a_1}^+}{\rightleftarrows}} E1 \tag{5}$$

where E1 is the initial C3 convertase. The C3 convertase then cleaves C3 into C3b, denoted Ab:

$$A + E_1 \underset{k_{E1}^-}{\overset{k_{E1}^+}{\rightleftarrows}} C1 \tag{6}$$

$$C_1 \xrightarrow{k_{E1}} E1 + Ab \tag{7}$$

where C1 is the enzyme-substrate complex, C3–$C3(H_2O)$Bb. This catalytic conversion of A to Ab can be inhibited by the inactivation of E1. This is represented as

$$E1 \xrightarrow{k_{d_2}} I \tag{8}$$

where I is the inhibited form of the enzyme. Otherwise, C3b can go on to form the MAC, be inhibited, or become a C3 convertase. The first two steps can be represented, respectively, as

$$Ab \rightarrow MAC \tag{9}$$

$$Ab \xrightarrow{k_{d_1}} I \tag{10}$$

where I is the inhibited form of C3b. The last step can be represented as

$$Ab \xrightarrow{k_2} B2 \tag{11}$$

where B2 now represents surface bound C3b and E1 represents the actual C3 convertase, C3b,Bb.

When an infection occurs, C3b binds on the membrane of the foreign agent and initiates the MAC. The final result is the elimination of the foreign cell. In the process of elimination, the byproducts, which in this case are the side chains like C3a, stimulate the production of C3. This final process is contracted and modeled as

$$Z + Ab \xrightarrow{k_{0A}} A + Ab \tag{12}$$

The number of microbes in a system is controlled by the rate of injection as well as the rate of reproduction. This is represented respectively as

$$Inf \xrightarrow{k_{inf}} Z \tag{13}$$

$$Z \xrightarrow{k_{rep}} 2Z \tag{14}$$

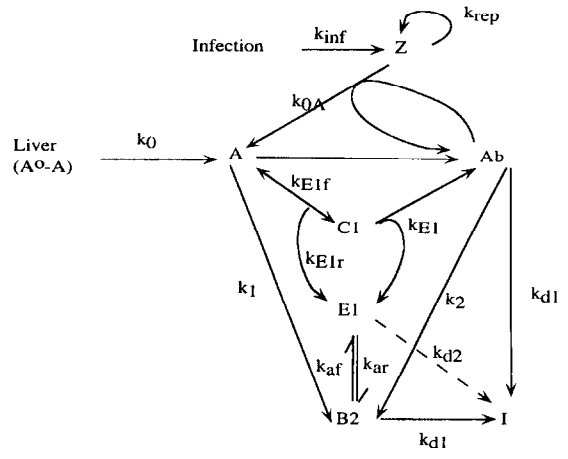

Fig. 2. A summary of the mechanisms in the minimal model.

Notice that the reproductive step is an autocatalytic step.

The minimal model of the activation of the alternative pathway of complement is summarized in Fig. 2. The mass action laws of the model are given by the following set of differential equations:

$$\frac{dZ}{dt} = k_{rep}Z + k_{inf} - k_{0A}Z\,Ab \tag{15}$$

$$\frac{dA}{dt} = k_0(A_0 - A) + k_{0A}Z\,Ab + k_{E1}^{-}C1 - k_1A - k_{E1}^{+}A\,E1 \tag{16}$$

$$\frac{dB2}{dt} = k_1A + k_2Ab + k_{a_1}^{-}E1 - k_{a_1}^{+}B2 - k_{d_1}B2 \tag{17}$$

$$\frac{dC1}{dt} = k_{E1}^{+}A\,E1 - k_{E1}^{-}C1 - k_{E1}C1 \tag{18}$$

$$\frac{dE1}{dt} = k_{E1}^{-}C1 + k_{E1}C1 + k_{a_1}^{+}B2 - k_{a_1}^{-}E1 - k_{E1}^{+}A\,E1 - k_{d_2}E1 \tag{19}$$

$$\frac{dAb}{dt} = k_{E1}C1 - k_2Ab - k_{d1}Ab \tag{20}$$

For this minimal model, steady states, sustained oscillations, period doubling route to chaos, as well as mixed-mode oscillations are observed when the values for the parameters and initial conditions used in the integration are comparable to experimental values [19,21,22]. Thus the simulations are geared toward maintaining physical accuracy. A further assurance arises from the fact that the results of the simulation fell within physiologically reasonable ranges. This means that concentrations were observed in the 10^{-6} M to the 10^{-3} M ranges. At the low end, 10^{-10} M is acceptable although it may not be detectable; concentrations lower than that are essentially negligible.

4. Contracted model of the alternative pathway

Steady state approximations are often used in the study of enzyme kinetics. In deriving the rate laws for kinetic mechanisms, it is typical to assume that the enzyme bound intermediates exist at steady state,

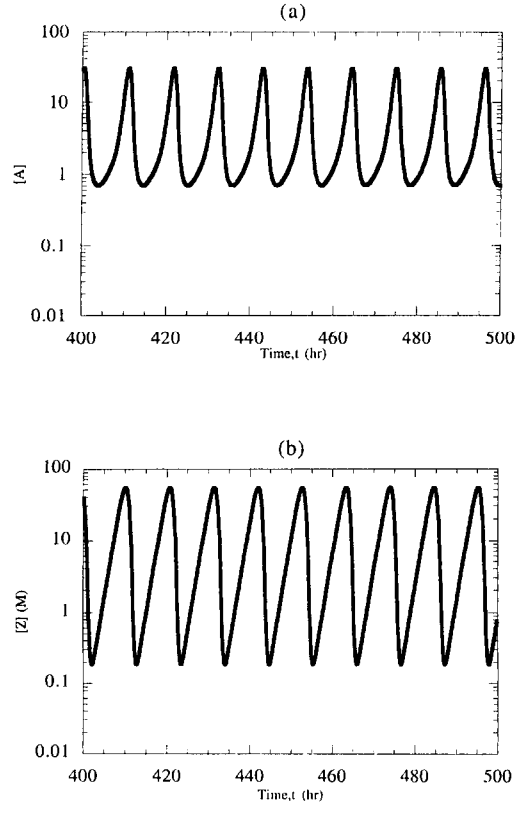

Fig. 3. Concentration vs. time plots for the minimal model when all the parameters are equal to unity. A represents C3 and Z represents the infectious agent.

ie., their rates of formation are equal to their rates of dissociation. The motivation for studying the effects of steady state assumptions in a mathematical model of a biochemical system is to determine whether or not one could reduce the number of variables under consideration. Should the simplified model exhibit similar activities as the original model, then the system would be easier to analyze in that fewer variables need to be taken into consideration.

The method typically employed when making steady state assumptions is the Briggs–Haldane. This is the steady state assumption as applied to the Michaelis–Menten model of enzyme catalysis. The Michaelis–Menten model of an enzyme catalyzed reaction involves the rate determining formation of an enzyme-substrate complex which dissociates to the product while regenerating the enzyme. The following is a schematic typical of Michaelis–Menten kinetics:

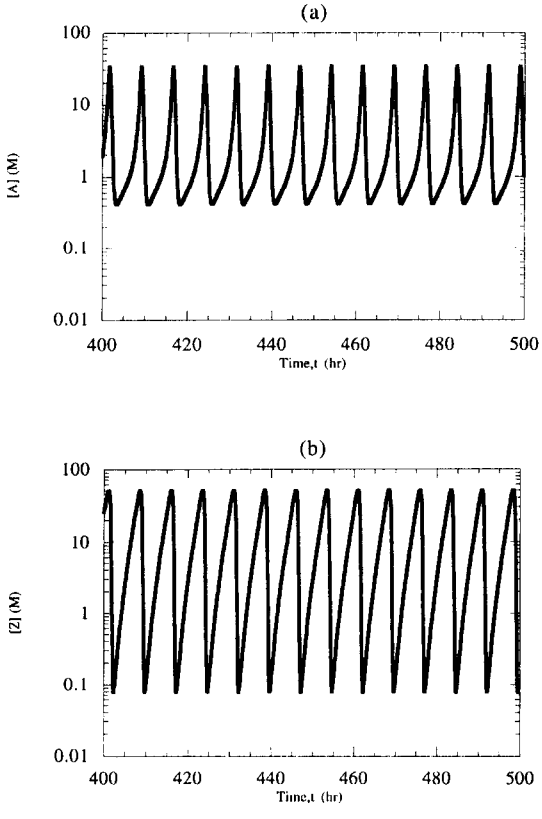

Fig. 4. Concentration vs. time plots for the contracted model when all other parameters are equal to unity. A represents C3 and Z represents the infectious agent.

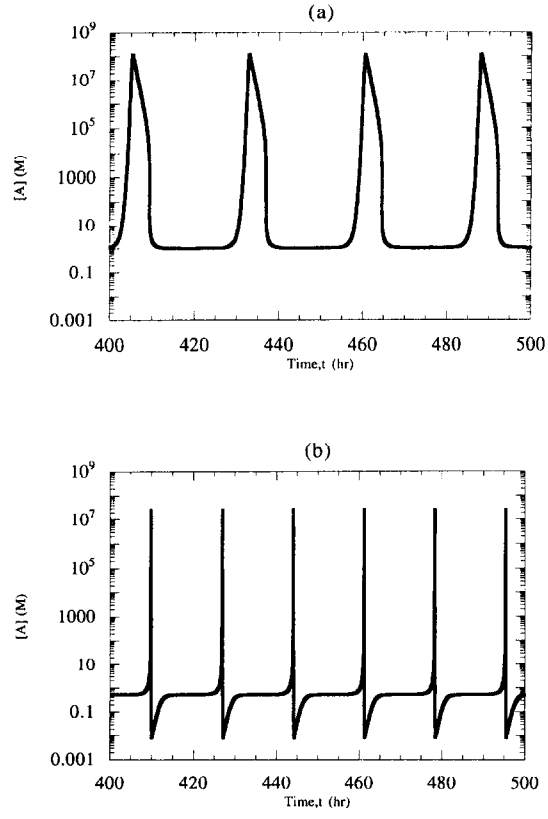

Fig. 5. Concentration vs. time plots for the (a) minimal and (b) contracted model when $k_{d_1} = 1000$ and all other parameters are set equal to unity.

$$E + S \underset{k_{-1}}{\overset{k^{+1}}{\rightleftarrows}} E + P \tag{21}$$

where E is the enzyme, S is the substrate, ES is the enzyme substrate complex, and P is the product.

A steady state approximation is made on the formation of C1, the enzyme substrate complex. This is a seemingly reasonable approximation since the physiological values for k_{E1}^{+} (3.6×10^{12} M^{-1} h^{-1}) and k_{E1}^{-} (3.6×10^{10} h^{-1}) are several orders of magnitude greater than k_{E1} (7.2×10^{6} h^{-1}), thus ensuring a steady concentration of C1 [22]. If the following substitution for C1 is made:

$$C1 = \frac{k_{E1}^{+} A E1}{k_{E1}^{-} + k_{E1}} \tag{22}$$

the mass action laws for the contracted model are given by the following equations [23]:

$$\frac{dZ}{dt} = k_{rep}Z + k_{inf} - k_{0A}ZAb \tag{23}$$

$$\frac{dA}{dt} = k_0(A_0 - A) + k_{0A}ZAb - k_{E1}\left(\frac{k^+_{E1}AE1}{k^-_{E1} + k_{E1}}\right) - k_1 A \tag{24}$$

$$\frac{dB2}{dt} = k_1 A + k_2 Ab + k^-_{a_1}E1 - k^+_{a_1}B2 - k_{d1}B2 \tag{25}$$

$$\frac{dE1}{dt} = k^+_{a_1}B2 - k^-_{a_1}E1 - k_{d_1}E1 \tag{26}$$

$$\frac{dAb}{dt} = k_{E1}\left(\frac{k^+_{E1}AE1}{k^-_{E1} + k_{E1}}\right) - k_2 Ab - k_{d_1}Ab \tag{27}$$

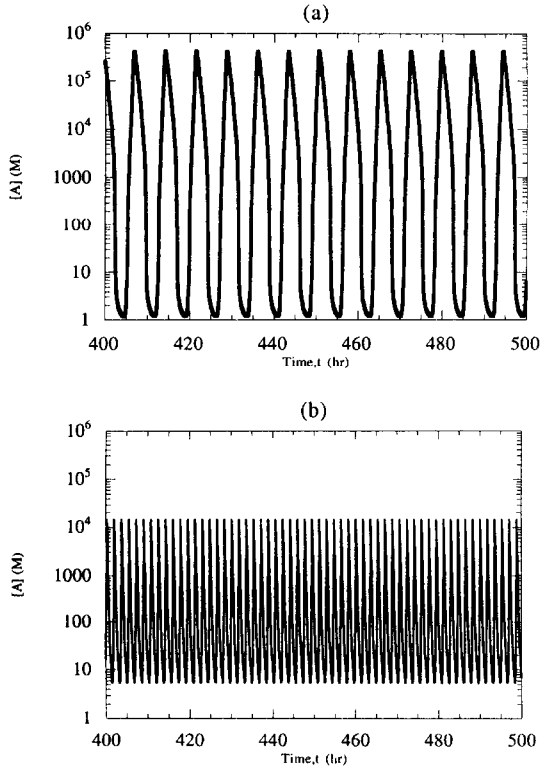

Fig. 6. Concentration vs. time plots for the (a) minimal and (b) contracted model when $k_{rep} = 10$, $k_{d_1} = 1000$ and all other parameters are set equal to unity.

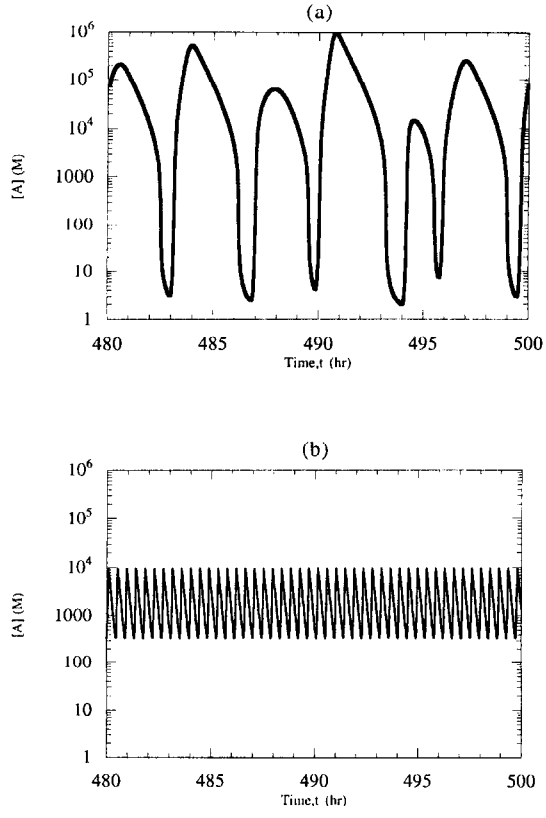

Fig. 7. Concentration vs. time plots for the (a) minimal and (b) contracted model when $k_{rep} = 100$ and $k_{d_1} = 100$ and all other parameters are set equal to unity.

The motivation behind studying the contraction of the minimal model of the alternative pathway of complement is to attempt to model the original six variable system by a five variable system. This would facilitate any attempt of an analytical study of the alternative pathway. For example, if the five variable system could be contracted further to a four variable system, then stability analysis could be applied to the four variable model, and the characteristic polynomial for the Jacobian of the four variable model would be a four order polynomial whose solution could be obtained in principle. A direct relation between the eigenvalues of the characteristic polynomial and the stability of the steady state solutions exists.

When making a steady state approximation, the

concentration of the enzyme substrate complex is assumed to be constant. This could become a problem when dealing with an enzyme cascade system since the concentration of E is constantly changing with time. Thus this change affects the concentration of ES. Also, implicit in the steady state approximation is that the concentration of the enzyme is negligible compared with that of the substrate, thus ensuring that the intermediate species dissociates. The freed enzyme can rapidly bind a nearby substrate molecule and maintain a constant intermediate concentration. This is generally a safe assumption when the amount of enzyme of a reaction remains constant. Again, in an enzyme cascade with a positive feedback mechanism, this assumption must be applied with caution since the concentration of enzyme can change appreciably with time.

4.1. Numerical analysis of the minimal and contracted model

Using PLOD 6.0 [24], numerical integration of both the minimal and the contracted models was carried out using the Gear algorithm [10]. The resulting data was compared for similarities and differences. Also we used INSITE [25] to obtain Poincaré sections.

First, we performed several integrations to determine the accuracy of integration necessary to ensure that the observed results would not be numerical artifacts. In these integrations, all parameters were set to one except for k_{d_1} which was set at 1×10^9. In addition, the initial conditions were set to one. We determined that an accuracy of 10^{-10} was sufficient to ensure no numerical artifacts.

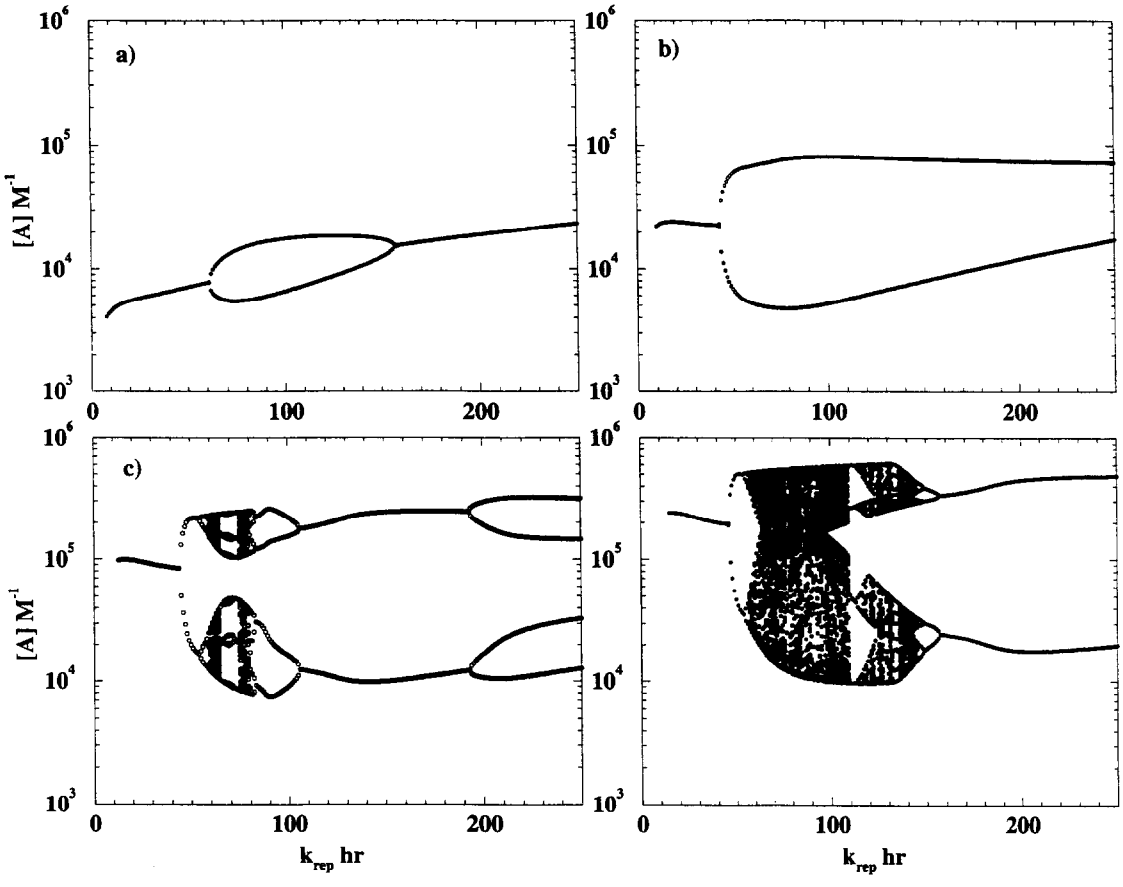

Fig. 8. Poincaré section defined by the maximum of A, varying k_{rep} for various values of k_{d_1}: (a) 10, (b) 25, (c) 50, and (d) 75, while holding the other parameters equal to unity.

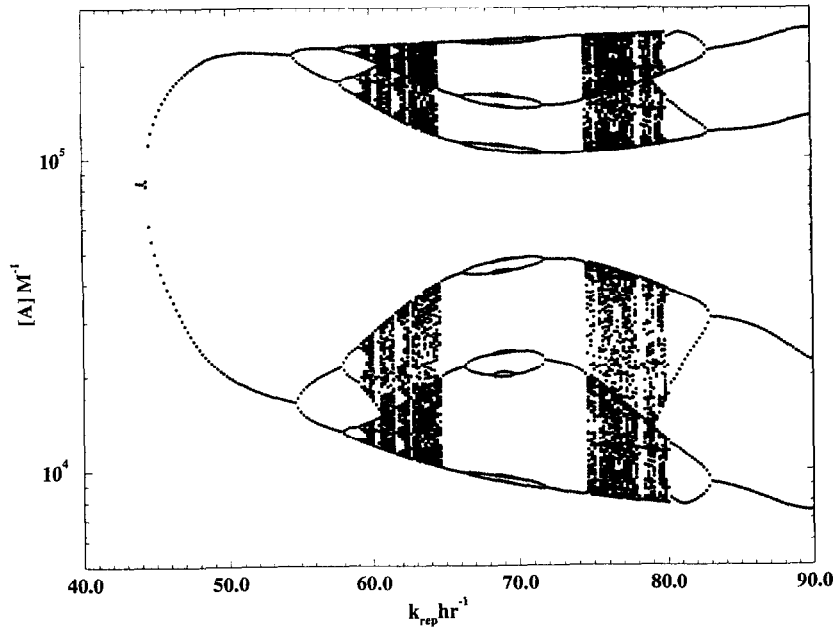

Fig. 9. Poincaré section defined by the maximum of A, varying k_{rep} when $k_{d_1} = 50$ and all other parameters are set equal to unity.

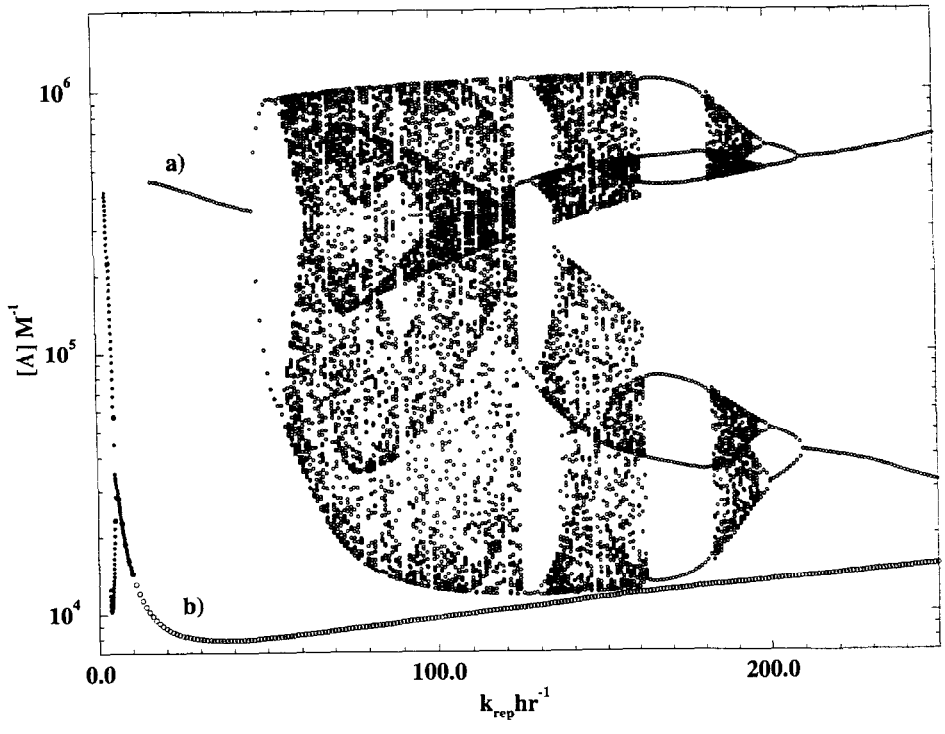

Fig. 10. Poincaré section defined by the maximum of A, varying k_{rep} when $k_{d_1} = 100$ and all other parameters are set equal to unity for the (a) minimal model and (b) contracted model.

Next, a set of runs which involved varying the deactivation parameter, k_{d_1}, were considerer while the other parameters were set to unity. Since k_{d_1} is the parameter which regulates the activity of the alternative pathway as modeled in both the minimal and contracted model, we expect to play a significant role in governing the complex dynamics of both models. Another motivation for varying k_{d_1} is that previous studies on this parameter have indicated it to be the key to the onset of period doubling [22,21]. The trend observed in the plots of B2, Z, A and Ab for both models was an increase in period and amplitude of oscillation with increasing k_{d_1}. Between the two models, the period of the contracted model was consistently smaller than that of the original model, but not significantly so. More significant, however, was the evolution of shape differences as k_{d_1} is varied. Notice from Figs. 3 and 4 that when k_{d_1} is one, the numerical trajectories of A, and Z, are, relatively speaking, similar in shape. However, as k_{d_1} was increased, differences arose in the two models. The behavior observed from the minimal model are characteristic of relaxation oscillation while those of the contracted model are not. The distinguishing feature of relaxation oscillations is the gradual accumulation of Z, which triggers a sharp increase in the concentrations of the cascade species, which causes a sharp decline in the concentration of Z, and the process repeats itself. The trajectories of the contracted model are spikes. Fig. 5 depicts the differences observed in the two models.

Varying k_{d_1} demonstrated that the responses of the two models are different. However, it did not seem as though either model was exhibiting any trends toward chaos. Thus another parameter, k_{rep}, was monitored. It makes sense to keep track of this parameter since it is related to the only autocatalytic step in the mechanism. Thus it should have some direct impact on the direction of the pathway. In addition, k_{d_1} was set to 100, a value at which the oscillations of the original and contracted model differed. The values of k_{rep} examined were 10, 100, and 1000.

For each value of k_{rep}, we found discrepancy between the minimal and the contracted versions in the frequency of oscillations. The contracted model oscillated with larger frequency than that of the minimal model. This is demonstrated by comparing

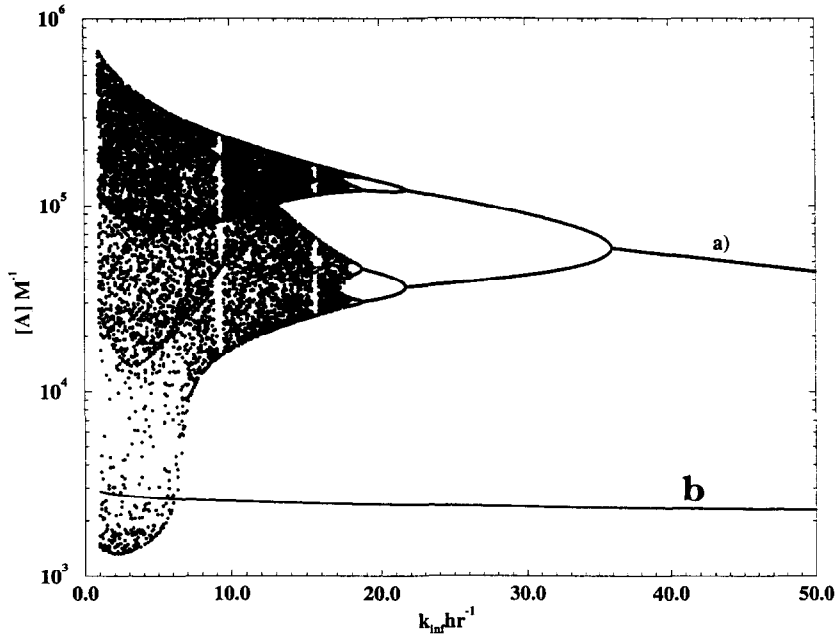

Fig. 11. Poincaré section defined by the maximum of A, varying k_{inf} when $k_{d_1} = 100$, $k_{rep} = 75$ and all other parameters are equal to unity for the (a) contracted model and (b) minimal model.

the minimal and contracted plots of A given in Figs. 6 and 7. Note also that frequency increases significantly with increasing k_{rep}. This is due to the fact that k_{rep} governs an autocatalytic step.

Poincaré sections defined for the maxima of A are generated in order to explore the chaotic region found in the minimal model. Fig. 8 depicts the Poincaré section as we vary k_{rep} from 0 to 250. The figures represent values of k_{d_1} equal to 10, 25, 50, and 75 and the values of all other parameters was 1. Chaos is seen in these diagrams when k_{d_1} is equal to 50 and 75. Fig. 9 is a magnification of the Poincaré section for k_{d_1} equal to 50. Fig. 10 is a comparison of the Poincaré sections as we vary k_{rep} and set k_{d_1} equal to 100, and all other parameters have value unity for the minimal and contracted model. As we can see, only oscillatory solutions appear in the contracted model, which means that the chaotic behavior of the minimal model is lost.

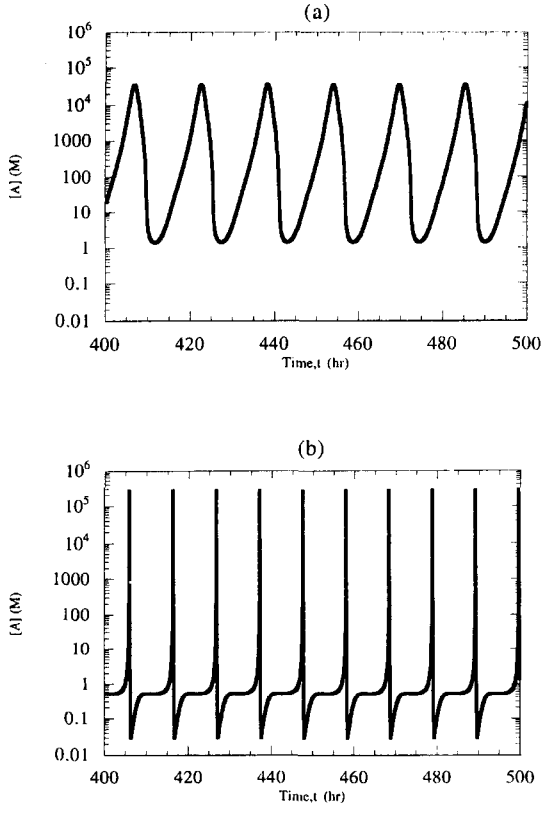

Fig. 12. Concentrations vs. time plots for the (a) minimal and (b) contracted model when $k_{inf} = 10$, $k_{d_1} = 100$, and all other parameters equal to unity.

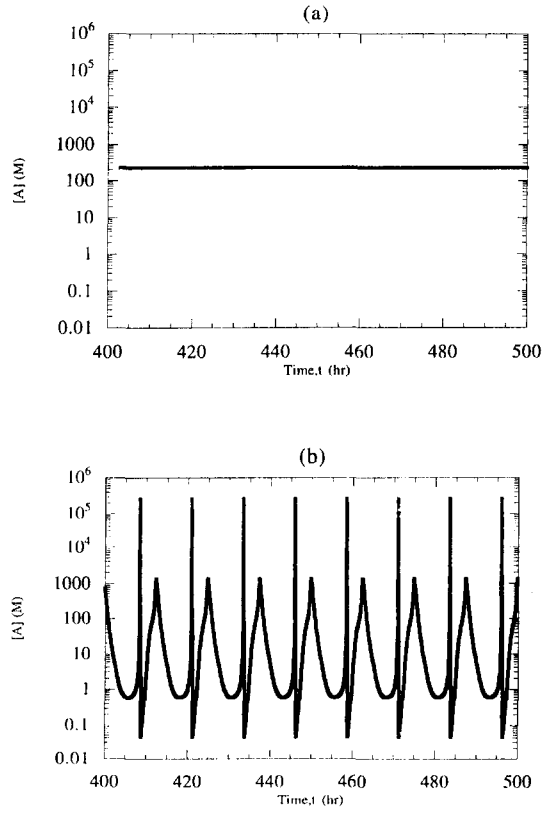

Fig. 13. Concentration vs. time plots for the (a) minimal and (b) contracted model when $k_{inf} = 100$ and $k_{d_1} = 100$ and all other parameters are equal to unity.

The final set of parameters involved varying k_{inf}. In this case, k_{inf} controls the rate of injection of Z, and thus should have some direct impact on the direction of the pathway. Consequently we set k_{rep} to 75, a value for which chaos is observed in the minimal model when k_{d_1} is 100, and all other parameters set to unity. For these values the bifurcation diagrams for k_{inf} show chaotic behavior in the minimal model, but oscillatory solutions in the contracted model (Fig. 11).

Finally we set $k_{d_1} = 100$ and consider $k_{inf} = 10$ in Fig. 12, and $k_{inf} = 100$ in Fig. 13 with the rest of the parameters equal to unity. The doubling of the period in the contracted model may suggest a chaotic behavior. Confirmation to this behavior is given by a

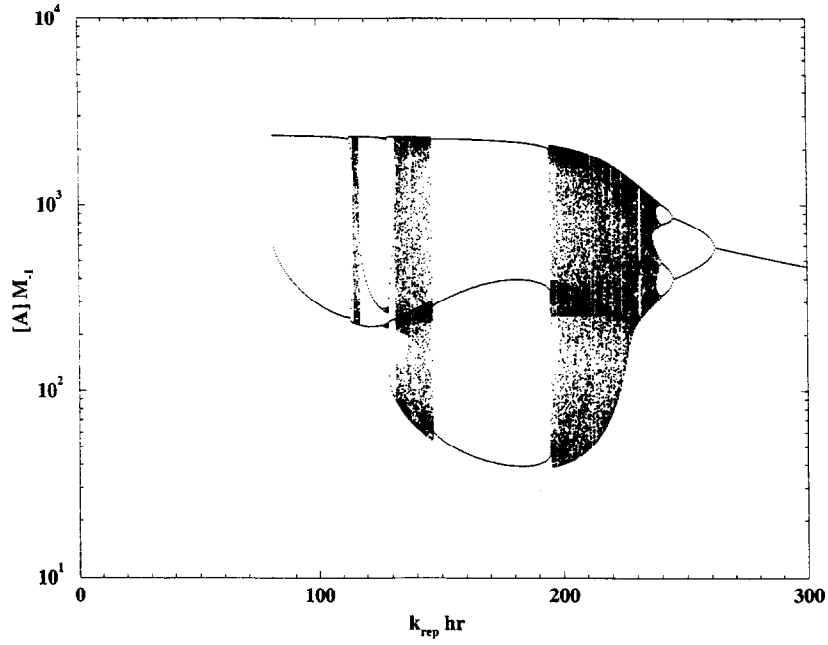

Fig. 14. Poincaré section defined by the maximum of A, varying k_{inf} when $k_{d_1} = 100$, and all other parameters are equal to unity for the contracted model. The minimal model shows a steady state and thus does not appear on the diagram.

Poincaré section as function of k_{inf} with k_{d_1} equal to 100 and all other parameters equal to one. In contrast we observed a simple steady state solution for the minimal model (Fig. 14).

5. Discussion

Although the present analysis was carried out for non-biological parameter values, where concentrations of even 1 M are extremely high for many biological systems, this study is designed only to compare the changes in dynamical behavior when the steady state approximations are used to contract the minimal model of the alternative pathway. To compare the dynamical behavior of the minimal model and the contracted model, we constructed several plots of the variable A. These plots of variable A are representative of the degree of activation of the alternative pathway since A represents the amount of C3 present.

The displayed chaotic behavior of the minimal model for certain values of k_{d_1} and k_{rep} is not seen in the contracted model for the same parameter space region. From the results involving k_{rep} we can conclude that the minimal and contracted model yield different dynamical behaviors. These different dynamical behaviors seem to indicate that the two representations of the alternative pathway may not be compatible.

The results from this study indicate that application of steady state approximation to enzymatic cascade systems may cause drastic changes in their dynamical behaviors. Thus, even in instances where it seems reasonable to use the steady state approximation in cascade mechanisms, contracted version can not be trusted.

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