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Mathematical Model of Two Isomeric Conformations for WASP Autoinhibition

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Abstract

The Wiskott-Aldrich syndrome Protein (WASP) has been implicated in many diseases including Wiskott-Aldrich Syndrome (WAS) and Buruli ulcer, but no mathematical model has been developed yet to describe the kinetics/dynamics of WASP. WASP is regulated by autoinhibition. In the autoinhibited complex, intramolecular interactions with the GTPase-binding domain (GBD) occlude residues of the C terminus that regulate the Arp2/3 actin-nucleating complex. Binding of Cdc42 to the GTPase-binding domain relieves the autoinhibitory contact between the GTPase-binding domain (GBD) and the C-terminal VCA region of WASP proteins and causes a dramatic conformational change, enabling its interaction with the actin regulatory machinery. Here we have developed a mathematical model that quantitatively describes WASP by two isomeric conformations, an active, largely unfolded conformation that is able to stimulate the Arp2/3 complex, and an inactive, folded conformation. The model invokes an intrinsic isomeric equilibrium constant L and an affinity constant C to control intramolecular contacts between the regulatory GBD and the activity-bearing VCA domain of the protein. The formulation is concentration-dependent based on steady-state equilibrium and conservation principles. By this approach we are able to quantify the fractional response of WASP against change in concentration of ligand. The model accurately predicts WASP autinhibition. The analysis confirms that WASP needs Cdc42 as an activator for maximal activation. In the absence of a ligand, WASP is regulated by the intrinsic isomeric equilibrium constant L . We also find that the stability of equilibrium of the model is affected by the Cdc42 affinity of WASP. The results further augment the understanding on the role of WASP in polymerization of actin filament and cytoskeletal rearrangement.

Keywords: Wiskott - Aldrich Syndrome Protein**,** autoinhibition, isomeric, conformation, receptor, ligand, enzyme, protein, binding**.**

1. Introduction

Wiskott–Aldrich Syndrome Protein (WASP) regulates the cytoskeleton in hematopoietic cells and thus plays a pivotal role in cellular locomotion. Intact cellular migration is critically important for the induction and regulation of the immune response. WASP deficiency causes the Wiskott–Aldrich Syndrome (WAS), a primary immunodeficiency with microthrombocytopenia, eczema and a higher susceptibility to develop tumors (Miria et. al 2013). Several distinctive abnormalities of T, B, dendritic cells; and phagocytes have been found in WASP-deficient patients (Recher 2012). Recent studies have also shown that the polyketide lipid toxin (mycolactone), the main virulence factor in the Buruli ulcer disease operates by hijacking the autoinhibitory mechanism of WASP leading to an uncontrolled polymerization of the actin filament. To understand the behavior and functions of WASP, we develop a mathematical model for WASP autoinhibition.

The model begins with enzyme-substrate kinetics proposed by Henry Michaelis and Menten (HMM). Later we introduce binding of WASP and its activators to model the pharmacokinetics and or pharmacology of WASP. The two time scales that results from HMM is not experimentally measurable therefore we adopt an approach to find fraction of bound ligand-receptor complexes. This way we are able to measure the fraction of bound WASP complex that can activate Arp2/3 complex to initiate polymerization of actin filament. The approach was first proposed by Briggs-Haldane (1925) for protein with one binding site. Before we develop the model for WASP and Cell division cycle 42 (Cdc42) binding, we give a brief introductory theory of equilibrium reactions.

1.1. Enzyme/receptor reaction kinetics

The complexity of biological and biochemical processes is such that the development of simplifying models and reaction schemes are often essential in trying to understand the phenomenon under consideration. For such models and reactions we should use reaction mechanisms which are plausible biochemically. Biochemical reactions are continually taking place in all living organism and most of them involve proteins called enzymes/receptors, ligands etc. The most important features of enzymes are: regulation, specificity on substrate and catalytic power. For example, haemoglobin in red blood cells is an enzyme and oxygen, with which it combines, is a substrate. enzyme kinetics mirrors some general types of reaction and

binding phenomena with their corresponding mathematical realization. Knowledge of these is essential in constructing the model for WASP autoinhibition and others to reflect specific known biochemical properties of a mechanism.

1.2. Chemical reaction equilibrium approximation: Henri-Michaelis-Menten (HMM) equation

Michaelis and Maud Menten (1913) proposed a reaction mechanism for the enzyme-catalyzed biochemical reaction based on experimental observations. In their model, an enzyme [E] reacts with a substrate [S] to form an intermediate complex [ES]. This intermediate complex breaks down not only to form back the reactants [E] and [S] but also the products [P] and [E].

In this study we refer to such a reaction scheme as enzyme/protein with one binding site.

Readers may find details in (Murray . (2001)). The reaction scheme can be written as:

$$[S] + [E] \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [ES] \xrightarrow{k_2} [P] + [E]$$

The evolution equations for the different species follow the law of mass action as:

$$\begin{cases} s_t = -k_1 se + k_{-1} c \\ e_t = -k_1 se + (k_{-1} + k_2) c \\ c_t = k_1 se - (k_{-1} + k_2) c \\ p_t = k_2 c \end{cases} \tag{1}$$

The initial concentrations are: $s(0) = s_0,\ e(0) = e_0,\ c(0) = p(0) = 0$. Where we have used small letters to represent the concentrations and $c = [SE]$,. $c_t = \dfrac{d[SE]}{dt}$. The last equation in (1) is uncoupled and provided the concentration (c) is known, we can write:

$$p(t) = k_2 \int_0^t c(t')dt' \tag{2}$$

From conservation of mass, since part of the enzyme is used up in the formation of the complex (c), adding the second and third equation of equation (1) we have:

$$e(t) = e_0 - c(t) \tag{3}$$

Let $$u = \frac{s}{s_0} c,\ v = \frac{}{e_e},\ \varepsilon = \frac{e_0}{s_0},\ K_m = \frac{k_{-1} + k_2}{k_1},\ K = \frac{K_m}{s_0},\ \gamma = \frac{k_2}{k_1 s_0},\ \tau = k_1 e_0 t \tag{4}$$

be dimensionless constants. Substitute (3) and (4) in equation (1), with initial conditions we have:

$$\begin{aligned} &u_\tau = -u + (u + K - \gamma)v \ \text{ and } \ \varepsilon \mathrm{v}_\tau = u - (u + K)v \\ &u(0) = 1,\ v(0) = 0 \end{aligned} \tag{5}$$

Using singular perturbation techniques to find the solution to equation (5) of the form $w(\tau, \gamma) = \sum_{n=0}^{\infty} \varepsilon^n u_n(\tau)$, we get the $0(1)$ order solution as:

$$v_0 = \frac{u_0}{u_0 + K},\ \ u_0(\tau) + K \ln u_0(\tau) = -\gamma\tau + 1 \tag{6}$$

Clearly the first part of the solution in (6) does not satisfy the initial condition in (5). In most biological application, where $0 < \varepsilon \ll 1$, inclusion of higher order terms ($0(\varepsilon^n),\ \ n = 1, 2, \ldots$) does not remedy the problem therefore, the

assumption that $\varepsilon = \frac{e_0}{s_0} \ll 1$ is reframed to include solutions near $\tau = 0$, (for $v(\tau)$).Introducing the new time scale: $\varphi = \frac{\tau}{\varepsilon}, \Rightarrow \varepsilon \to 0,\ \varphi \to \infty$. Substituting into (5) and simplifying, we obtain the solution:

$$U_0(\varphi) = C, \Rightarrow U_0(\varphi) = 1$$
$$V_0(\varphi) = \frac{1}{1+K}[1 - \mathrm{e}^{-(1-K)\varphi}] \tag{7}$$

The matching conditions are given by:

$$0(1) : \lim_{\varphi \to \infty}[U(\varphi,\varepsilon), V(\varphi,\varepsilon)] = \lim_{\tau \to \infty}[u(\tau,\varepsilon), v(\tau,\varepsilon)], \forall \varepsilon \tag{8}$$

The two time scales gives an inner and outer solution as follows:

$$u(\tau,\varepsilon) = u_0(\tau) + 0(\varepsilon), \tau \geq 0$$
$$v(\tau,\varepsilon) = \begin{cases} \underbrace{V_0(\varphi) + 0(\varepsilon), \quad 0 < \tau \ll 1}_{\text{Inner solution}} \\ \underbrace{v_0(\varphi) + 0(\varepsilon), \quad \tau \gg 1}_{\text{Outer solution}} \end{cases} \tag{9}$$

Remark: In the solution above the assumption that the initial concentration of the substrate-enzyme complex equals zero (c(0) = 0) is a mathematical interpretation since in reality life (protein and enzyme reaction) is a continuous process and such complexes cannot be zero.

Secondly in biological problems, the rapid change in the substrate-enzyme complex for the model discussed above in dimensionless and for dimensional times are so small that they are experimentally not measurable. Thirdly we have assumed in the discussion above that $\varepsilon \approx 0$, and therefore mathematically $\varepsilon v_t \approx 0$. The question is what happens if the ratio of enzyme to substrate (e_0 / s_0) is not so small? This was studied by De Boer and Perelson (1994) for a situation involving T-cell proliferation in response to an antigen. Therefore since it is our aim to measure the concentration of WASP protein that binds to GTPases-Cdc42 concentrations in real time, this method cannot be used.

In this study we use a general approach to investigate a reaction system of enzyme/receptor and substrates binding without any assumption on the enzyme substrate ratio. The approach can lead to the measurement of the fraction of concentrations of all the species in real time.

1.3 Quasi-Steady State Assumption

In the derivation of the HMM equation it was assumed that the formation of the complex [ES] was very fast, such that in a short time, it was in instantaneous equilibrium with the substrate [S] Therefore, $k_1, k_{-1} \gg k_2$ and $k_1 es = k_{-1} c$ from the first equation in (1). Applying the law of detri we can write an expression for the fraction of the complex (c) as

$$c = \frac{e_T s}{k_d + s} \tag{10}$$

where k_d is the equilibrium dissociation constant and $e_T = e + c$ is the total concentration of enzyme (bound and unbound). An alternative hypothesis was suggested by Briggs and Haldane (1925).They proposed that if the enzyme is present in catalytic amounts ($e \ll s$), then shortly after mixing [E] and [S], a steady state is established in which the concentration of [ES] remains essentially constant with time, thus $c_t = e_t = 0$. Then from the second or third equation in (1) we can again write the fraction of the complex (c) as:

$$c = \frac{e_T s}{s + K_m} \tag{11}$$

Equations (10) and (11) become equal only if $k_{-1} \gg k_2$. We can now use equation (11) to obtain an implicit solution for the substrate (s)

$$s_t = \frac{-qs}{s + K_m}, \quad q = k_2 e_T \tag{12}.$$

The function $\frac{s}{s + K_m}$ is a saturation function which saturates to unity as $s \to \infty$. The solution for (12) is given by:

$$s(t) + K_m \ln(s) = qt + A \tag{13}$$

Where $q,$ and A are constants to be determined. From equations (10), (11), and (12) we notice that enzymatic reactions do not follow the law of mass action directly. As the concentration of the substrate is increased, the rate of the reaction increases only to a certain extent, reaching a maximal (saturation) reaction velocity at high substrate concentration. This is in contrast with the law of mass action which, when applied directly to the reaction with the enzyme predicts that the velocity increases linearly as the substrate concentration.

1.4 Protein with two binding sites

A protein may have more than one (active or inactive) binding site. When a ligand is bound to the protein, there will be a conformational change in the receptive unit. The binding of a ligand to one site of the protein may or may not influence the binding of another ligand to a second site on the receptive unit. The mechanism of protein with two binding sites is shown in Fig. 1.

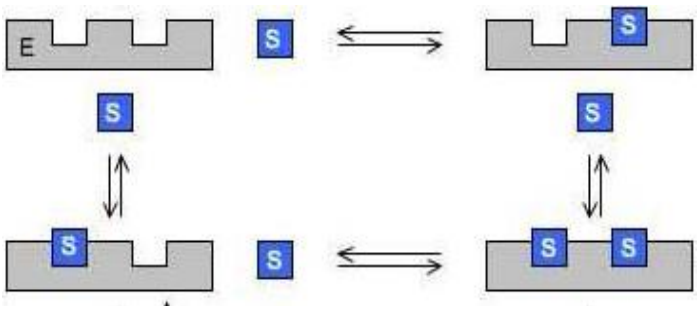


Figure 1, Mechanism of protein with two binding sites

For the mechanism above, if the binding is independent, the fraction of bound enzyme- protein complex is given by:

$$F_{in} = \frac{2e_T s}{k_d + s} \tag{14}$$

If the binding of the ligand to the protein is mutually inclusive (cooperative) where $k_j = \beta k_{j-1}, \quad \beta < 1$ and $k_j, \quad j = 1, 2$, are reaction rates and $k_j > k_{j+1}$. The fraction of bound enzyme-substrate complex is given by:

$$F_{dep} = \frac{e_T s^2}{\beta k_1^2 + s^2} \tag{15}$$

Note that equation (14) is twice (10), (the equation for protein with one binding site).

For the purpose of our study we give a brief introduction to the internal structure and function of the eukaryotic cell.

1.5 Introduction to dynamics of the actin cytoskeleton

The actin cytoskeleton is implicated in numerous cellular processes and more than a hundred actin-related proteins have evolved in eukaryotic cells to regulate the actin cytoskeleton in both space and time (Siripala and Welch 2007).One of the most fundamental and abundant protein to life and death is the actin in eukaryotic cells. Cells harness various actin binding proteins to build varied cellular structures and utilize the force generated by actin polymerization to drive these diverse processes. It is not surprising that toxic substances, bacteria and viruses have evolved mechanisms to interrupt or hijack and usurp the host actin machinery (eg. cytoskeleton) to serve their own needs during infection. Toxins and pathogens often target

the host actin cytoskeleton as a means to facilitate intimate attachment to host membranes, mediate their entry into host cells (Munter, Way et al. 2006; Stevens, Galyov et al. 2006).

Cells regulate actin cytoskeleton dynamics in response to extracellular stimulation. These signaling pathways modulate actin assembly and disassembly by switching on the Rho family. During actin assembly, the activated Rho GTPase Rac and Cdc42 stimulate the actin nucleation and branching factor Arp2/3 complex via members of the Wiskott - Aldrich syndrome Protein (WASP) family (Jaffe and Hall 2005, Hall 1998). On the other hand, GTP-Rho acts directly on formin proteins that nucleate and elongate actin filament cables, which are key structures for cytokinesis and myosin-driven transportation (Bruce et. al. 2008, Pollard and Cooper 2009).

1.6 Function of the VCA domain in WASP

WASP family proteins play a major role in regulating actin dynamics in cells. They are defined by a VCA (Verprolin homology region, Central hydrophobic region, Acidic region) domain. This protein family consists of WASP, N-WASP (neuronal WASP), WAVE (WASP family verprolin homolog) isoforms 1-3, and WASH (WASP/Scar homolog). They all share similar C-terminal VCA domains which are required for the biochemical activity of promoting actin polymerization by nucleation and branching factor Arp2/3 complex. Nearly all the WASP family, differ in their N-terminal domain organization, indicating that each member has distinct cellular localizations, modes of regulation, and biological functions.

WASP is composed of an N-terminal, a basic region (B), a GTPase binding domain (GBD) and a C-terminal VCA region. The VCA is the activity-bearing domain of WASP, whereas the other N-terminal domains mainly serve regulatory functions. WASP spatially and temporally coordinates numerous signal inputs via its various regulatory domains to give a specific functional output through the VCA that turns on the actin nucleating Arp2/3 complex. The major nucleation promoting activity of WASPs VCA is modulated by its N-terminal and the GBD. These regions receive or engage with various singling molecules to link extracellular stimulation to intracellular actin machinery. An important feature of WASP is its allosteric effect. The major activities of WASP reside in the VCA, which coordinates with Arp2/3 complex to nucleate actin filaments (Fig. 2).

1.7 WASP is autoinhibited via intramolecular interactions between GBD and VCA domains

WASP alone is autoinhibited because the activity bearing VCA is masked by the GBD. The VCA binds to the GBD, but this interaction can be weakened by addition of activated Cdc42 that binds to the GBD (Miki, Sasaki et al. 1998; Kim, Kakalis et al. 2000). The activity of WASP in actin assembly is enhanced by the presence of activated Cdc42 as will be seen in our model. WASP is autoinhibited via intramolecular interactions between their GBD and VCA. Binding of activated Cdc42 releases this inhibition and allows the VCA to bind Arp2/3 complex to initiate actin filament.

Research works from (Kim, Kakalis et al. 2000; Panchal, Kaiser et al. 2003) reports on the nuclear magnetic resonance (NMR) of actin polymerization assays shows that this interaction sequesters the C region of the VCA and blocks residues needed for Arp2/3 activation, thus inhibiting WASP to bind and activate Arp2/3 complex. The question is how this autoinhibition is achieved. WASP exists in two conformations: folded (inactive) and unfolded (active) (Mathias et. al. 2001)

1.8 The Rho-family GTPase Cdc42 releases WASP autoinhibition

The works of (Lamarche, Tapon et al. 1996; Symons, Derry et al. 1996) report that biochemical assays in combination with column chromatography show that Cdc42 binds to the WASP GBD in a nucleotide-dependent manner, with a high affinity in the GTP state, and this interaction links Cdc42 to the actin cytoskeleton in cells. Cdc42 competes with the VCA for binding to the GBD (Miki, Sasaki et al. 1998). Importantly, this binding interaction is required for stimulation of WASP activity in actin assembly. In the active state, the GBD-VCA is largely unfolded and the VCA is readily able to bind and activate Arp2/3 complex; whereas in the closed and inactive state, interactions between the GBD and the VCA blocks the accessibility of the VCA to Arp2/3 complex.. Binding of activated Cdc42 to the GBD shifts the equilibrium to the open state and globally destabilizes the autoinhibited fold, hence releasing the VCA and activating WASP to stimulate actin assembly by Arp2/3 complex (Buck, Xu et al. 2001; Kim, Kakalis et al. 2000; Abdul-Manan, Aghazadeh et al. 1999). The affinity of WASP is 500 to 1,000-fold higher for GTP-Cdc42 than for GDP-Cdc42, but also the efficiency of WASP activation is higher for GTP-Cdc42 than for GDP-Cdc42. Therefore the GTP-Cdc42 functions as a full ligand (agonist).

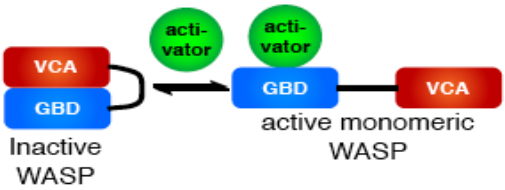


Figure 2: Autoinhibitory equilibrium of WASP

Dynamic rearrangements of the actin cytoskeleton are an integral part of many cellular processes including migration, adhesion, establishment and maintenance of polarity, and vesicle trafficking but defects in cytoskeletal structure, contribute to a variety of diseases, including cancer, developmental disorders and immunodefficiencies.

2. Mathematical model

Our model will be a formulation of concentration-dependent steady-state equilibrium, based on conservation principles. This formulation approach will mean that the receptive unit of interest

(eg. all activated receptors) are expressed as fractions of the total receptive units.
The equilibrium approach is employed to allow us quantify both binding and functional response of receptors against a change in concentration of the ligand, or as fractional response of receptors against a change in concentration of the ligand. Obviously in biochemical reaction, just because there is a conformational change in the receptor unit when a ligand binds, this conformational change is not necessarily the one which activates (or inhibits) the receptive unit for function. Meanwhile since theories on binding and function have many overlapping and identical expressions, their analysis are easily confounded (Bindslev 2008). Two constants, an isomeric equilibrium constant L and an affinity constant C are introduced to control intramolecular contacts (equilibrium) between the regulatory GTPase binding domain (GBD) and the activity-bearing VCA domain of the protein.

We apply the law of reciprocity which states: "if binding affects activation, then activation must affect binding" (Colquhoun 1998). This law allows us to formulate reversible reactions at equilibrium. The model is a formulation on a careful study of various researches in the literature and parameters are chosen from known and tested experiments (Mathias et. Al 2001, Laure G. et.al 2013, Luke and Brad 2013, Brain et. al. 2012)

2.1 Two isomeric conformations of WASP exhibits autoinhibition in a concentration dependent model

WASP is known to control the polymerization of actin filament and the cytoskeleton rearrangement of eukaryotic cells. In the reaction scheme below, WASP is the receptive unit (R) and GTPase Cdc42 is the ligand (A). As mentioned above, WASP (R) can exist in two conformations: active unfolded conformation (denoted R^*) and an inactive folded conformation (denoted T). The idea of using R and T is borrowed from the pioneers in this area of research Wyman (1948), Allen et. al (1950), Watanabe (1952), Blum (1955), Glynn (1955) and Monod et. al (1965). In these notation they meant that protein had to relax (R-state) in order to bind substrate and in the tense (T-state) it is inactive. Note that this does not mean the T-state isomeric conformation does not bind but rather it is inactive. We denote the active part of the WASP protein as R^* to make it different from the receptor WASP protein (R).

In the model these two isomeric conformation will constitute the two conformations/states of WASP protein (T or R^*). The ligand (A) can bind to either sides of R independently or simultaneously (mutually inclusive). Let us denote the binding of A to the left side of R (ie. A R) as TA, and the binding of A to the right side of R (ie. R A) as R^*A. Here binding of a ligand to the left of R is assumed to prevent activation of the receptive unit, therefore the receptor conformations AR and AR^*A are possible but not active. The AR^*A, is the complex of a receptive unit with a ligand bound simultaneously to its left and right sites and it is assumed non-active. In the scheme only the R^*A and R^*, are active conformations and ready to undergo the change to activate Arp2/3. This allows us to model auto-inhibition of the WASP on Cdc42 binding. The model developed here follows the scheme:

$$\text{Ligand} \longrightarrow \text{WASP} \longrightarrow \text{ARP complex} \longrightarrow \text{Actin filament}$$

For an equilibrium reaction in a closed system, the sum of all receptive conformations, bound and unbound (R_{tot}) is fixed. There will be a fixed number of bombardments of a finite number of receptive sites by ligand molecules with varying intensities as the ligand concentration varies. In this study, keep in mind that the model assumes a finite number of receptive units, while the ligand source is inexhaustible. By this finite number of receptive units we are able to quantify the fraction of bound ligand-receptive complexes at varying ligand concentration. Note that this adsorption process is saturable because there is a limited number of binding sites. The process of binding a ligand to its receptive unit is a chemical process theorized for equilibrium (Langmuir 1918). The proposed reaction mechanism for WASP auto-inhibition is shown below

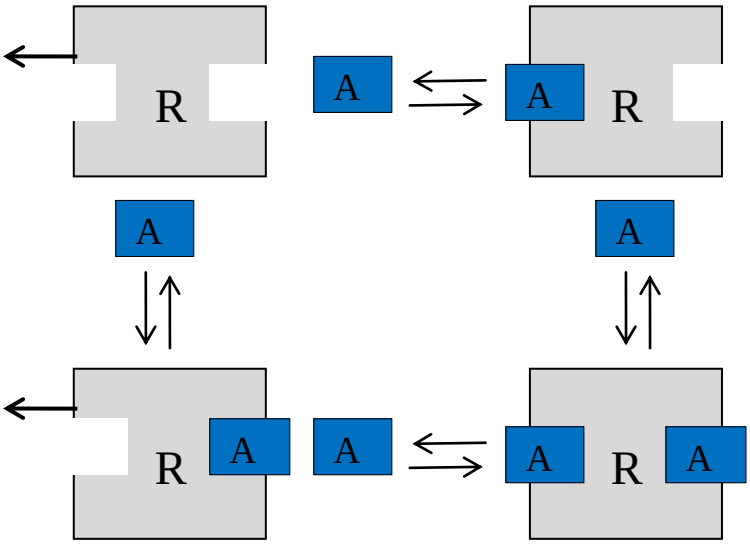


Figure 3 Reaction mechanism for Cdc42 binding with WASP protein

The reaction scheme for the reaction mechanism shown above can be written as:

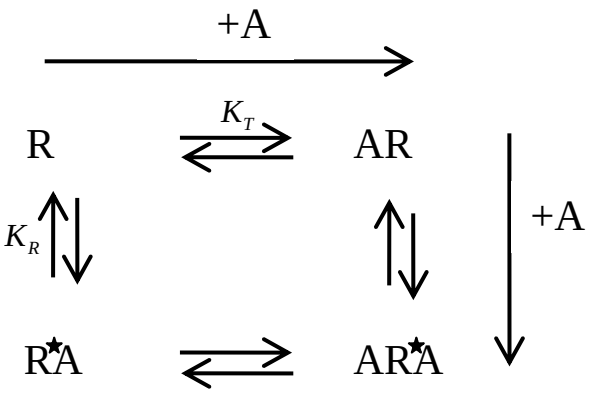


Figure 4 Reaction scheme for Cdc42 and WASP binding

We define the following receptor conformations and system constants**:**

$$L = \frac{[T_0]}{[R_0^*]},\ C = \frac{K_R}{K_T}\ ,\ S = \frac{[A]}{K_R},\ [\mathrm{ARA}] = R^*\left(\frac{[A]}{K_R}\right)\frac{[A]}{K_T} \tag{16}$$

$$[RA] = \frac{[R^*][A]}{K_R},\ [\mathrm{AR}] = \frac{[T][A]}{K_T} = \frac{L[R^*][A]}{K_T} \tag{17}$$

where L is an intrinsic isomeric equilibrium constant that describe the equilibrium of R^* and T in the absence of a ligand, [ARA] is a result of mutually inclusive binding (co-operativity), C is the affinity constant and also called the cooperative factor in this model. [R^*A] and [AR] are both receptor complexes with different equilibrium dissociation constants K_R and K_T respectively. We will drop the concentration symbols ([]) for convenience. There will be three scenarios of fractional-activation function responses, first will be termed self activation, second is activation induced on Cdc42 binding and thirdly the complete auto-inhibition of the WASP protein (sum of first two). We consider only the last two.

2.2 Scenario One: fractional activation induced on Cdc42 binding

Functionally, a quantitative measure of the fraction of WASP protein in the active conformation induced on Cdc42 binding in the reaction scheme is given below. Note that in the model we have assumed that in basal form WASP protein has an active part (R^*).

$$\frac{f_r}{R_{tot}} = \frac{R^*A}{R^* + R^*A + AR + AR^*A + LR^*} \tag{18}$$

Substituting equations (16) and (17) into (18) gives

$$F_r = \frac{S}{K_R + S + L + LSC + S^2C} \tag{19}$$

$F_r = \frac{f_r}{R_{tot}}$ and $R_{tot} = R^* + R^*A + AR + AR^*A + LR^*$ and S is a normalized concentration of the ligand (A).

2.3 Scenario Two: Self and induced autoinhibition of WASP protein on Cdc42 binding

The total fraction of WASP protein in active conformation is given by:

$$F_r = \frac{([free - R^* - state] + [bound - R^* - state])}{Total\ (R^* \text{ and } T - state)}$$

Making the substitutions gives:

$$F_r = \frac{1 + S}{1 + S + L + LSC + CS^2} \tag{20}$$

Equation (19) is the fraction of active conformation of WASP induced on Cdc42 binding whiles equation (20) shows the total fraction of active conformation of WASP protein. We notice from equation (20) that in the absence of a ligand ([A] = 0),

$$F_{r(0)} = \frac{1}{1 + L} \tag{21}$$

Secondly at very high concentration of the ligand $[A] \to \infty$, we have:

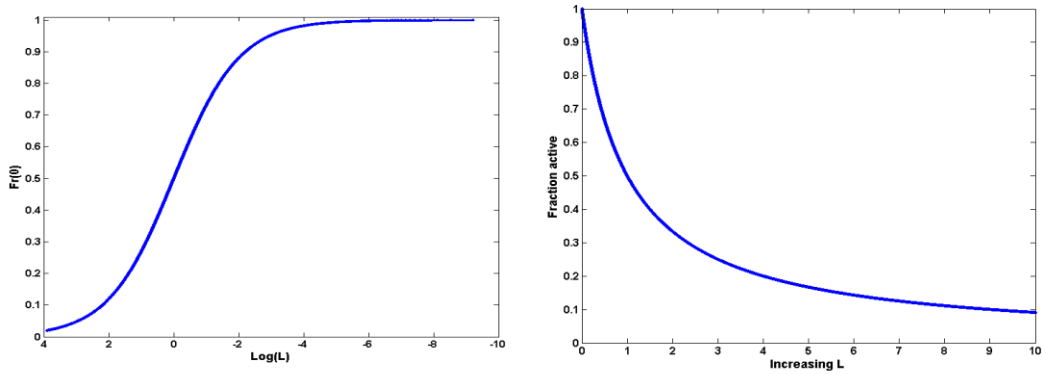
$$F_{r(sat)} = \frac{1}{1 + C(L + 1)} \tag{22}$$

$F_{r(0),}$ and $F_{r(sat)}$ are the active fractions of WASP in the absence of a ligand and at saturating concentration of the ligand respectively. Equation (21) implies in the absence of a ligand the WASP protein is regulated by the isomeric equilibrium constant L (it determines the stability of the reaction). As $L \approx 0$, ($T \ll 1$) steadily from one, the fraction of active conformation of the WASP

(f_r) and (R_{tot}) equalize and no binding occurs ($F_{r(0)} = 1$), there is saturation (Fig.5a). The reverse is also true if L rises steadily as R^* deceases and we have all T conformation (ie. $L \to \infty$). $F_{r(0)}$ decrease asymptotically, thus inhibition of the protein or inactive conformation of the protein (Fig. 5b).

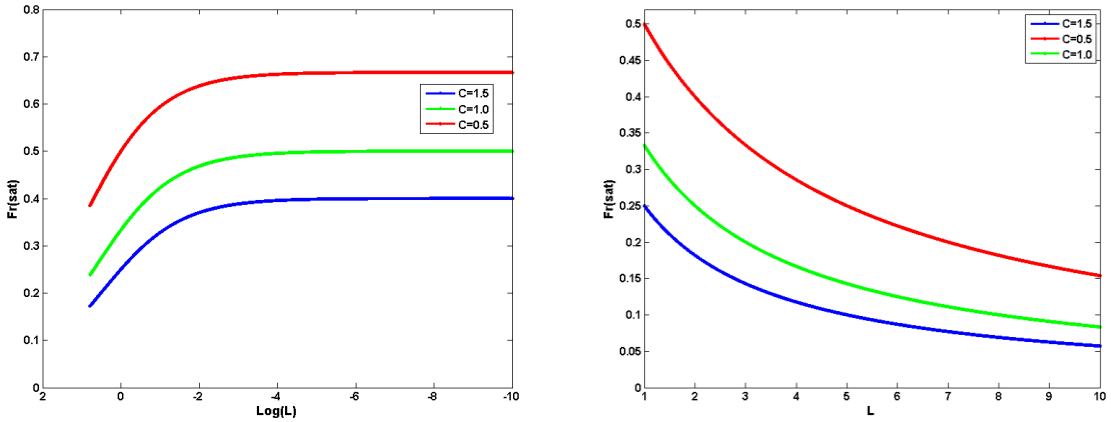
At saturating concentration of the ligand, the fraction of active conformation of WASP protein depends on affinity constant C and the isomeric equilibrium constant L .If $C<1$ the affinity of ligand (A) to R^* , is increased as L decreases asymptotically, hence the fraction of active conformation increases (Fig. 6a). The reverse is true for increasing C.

As L becomes large (all T-state) the fraction of active conformation of WASP protein decreases asymptotically (Fig. 6b). Note that the asymptotic decrease in the plots (Fig.5b) and (Fig.6b) show inhibition.



(a) (b)

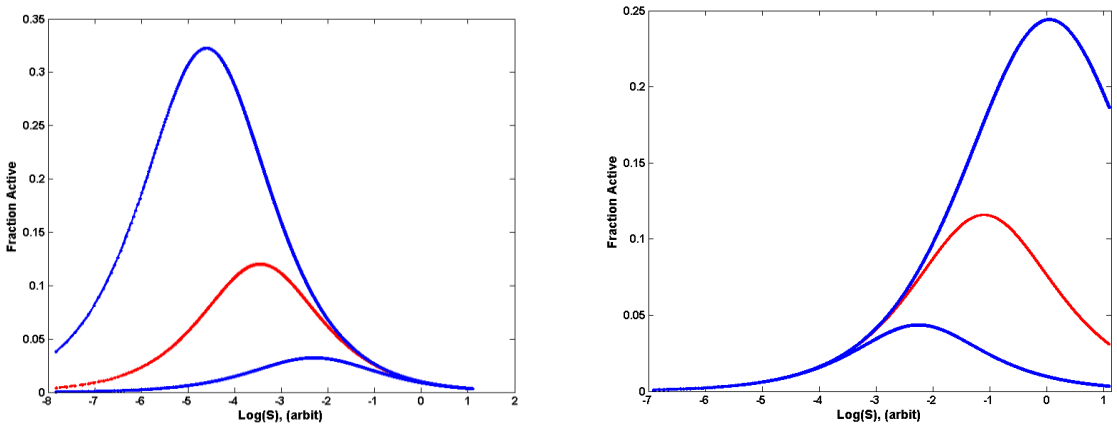
Figure 5.Fraction of the free WASP proteins (Fr(0)) in the active conformation as a function of L. (a) L < 1, (b) L > 1



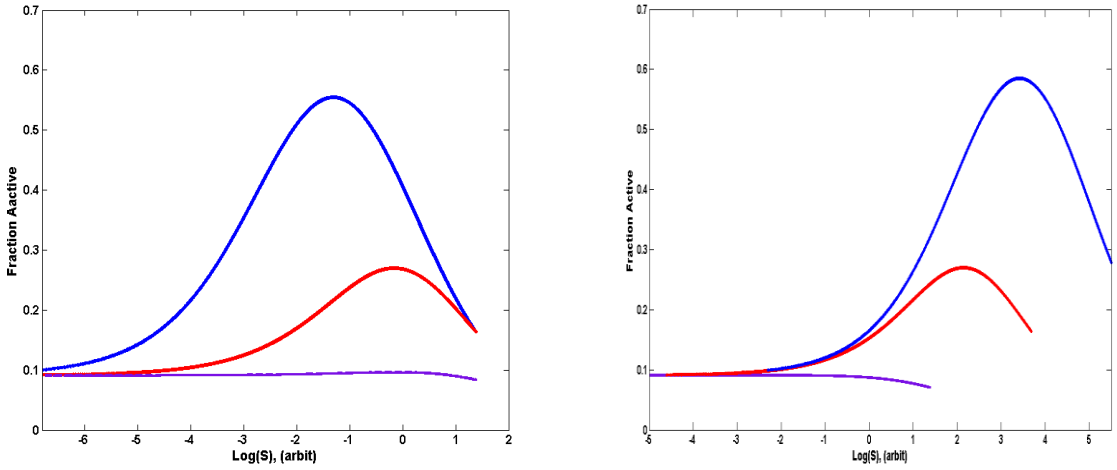
(c) (d)

Figure 6 Fraction of the saturated WASP proteins (Fr(sat)) in the active conformation as a function of L. showing the effect of C (a) L < 1, (b) L > 1.

Equations (19) and (20) are plotted below:



(a) (b)



(c) (d)

Figure 7 The plot shows Fraction of Active WASP protein complex vs Ligand concentration (S).

In Fig 7(a) the equilibrium dissociation constant is in the range $10^{-2} \le K_R \le 1$ in steps of 10, whiles K_T = C = L = 1. The highest fraction of active WASP complex corresponds to $K_R = 10^{-2}$ and decreases as K_R increases. It is observed that there is activation and inhibition of the WASP protein as the concentration of the ligand increases. This implies that in ligand- receptor binding where the protein can exist in two states (active and inactive), if the absorption rate is greater than the desorption rate, there will be an increase in the fraction of active WASP complex (ready to activate Arp2/3 complex) up a certain maximum depending on the parameters $L, C, K_R,$ and K_T, any further increase in the ligand concentration inhibits the protein. It also means that if all active sites of the WASP protein are bound, any additional concentration of ligand inhibits the protein.

In Fig 7(b) K_T is varied in the range $10^{-2} \le K_T \le 1$ in steps of 10 whiles KR = C = L = 1. Here the highest fraction of active WASP complex corresponds to $K_T = 1$ and decreases in that order. If $K_T \ge 1$ it implies desorption of the inactive complex of the WASP is greater than absorption. Therefore the fraction of active WASP complex increases though there is a delay and the curve shift to the right.

Fig. 7(c) and Fig. 7(d) are plots of equation (20). Notice the rise in the fraction of Active conformation of the WASP complex in complete auto-inhibition of the WASP protein. The fraction of active conformation of WASP complex in Fig. 7(a), increases by approximately 27% in fig. 7(c) The model above is not limited to autoinhibition of WASP protein but can also predict the models of Haldan's (1930), Laidler and Hoare's (1949). If the protein exist in only one state, then $L = 0$ and the model can be used to predict autointervention one state binding.

2.4 Binding

We derive the equation for a concentration-binding regime. Here receptive units with two bound ligands count twice. We formulate this as the fraction of bound receptive units:

$$F_R = \frac{R^*A + AR + 2AR^*A}{R^* + LR^* + R^*A + 2AR^*A} \tag{23}$$

Making substation into equation (23) simplifies to:

$$F_R = \frac{A}{A + K_R \dfrac{(1+L)}{\left(1 + LC + 2\,A/K_T\right)}} \tag{24}$$

It can be observed that equations (24) and (20) are different. Equation (20) is the fraction of active conformation of the receptor whiles (24) is the fraction of bound conformation of the receptor. Similar results of equation (24) are given by Laidler - Hoare (1949) and Haldane (1930 and 1956) for their one state auto-regulation scheme where $L = 0$.

Existing model in the literature is given by:

$$\frac{\text{Occupancy}}{\text{total}} = \frac{S}{S + \dfrac{K_{ss}}{1 + 2\,S/K_{is}}} \tag{25}$$

where $K_R = K_{ss}, K_{is} = K_T, S = A,$ and $F_R = \dfrac{\text{Occupancy}}{\text{total}}$.

The plots of equation (24) and (25) are shown below for varying parameters.

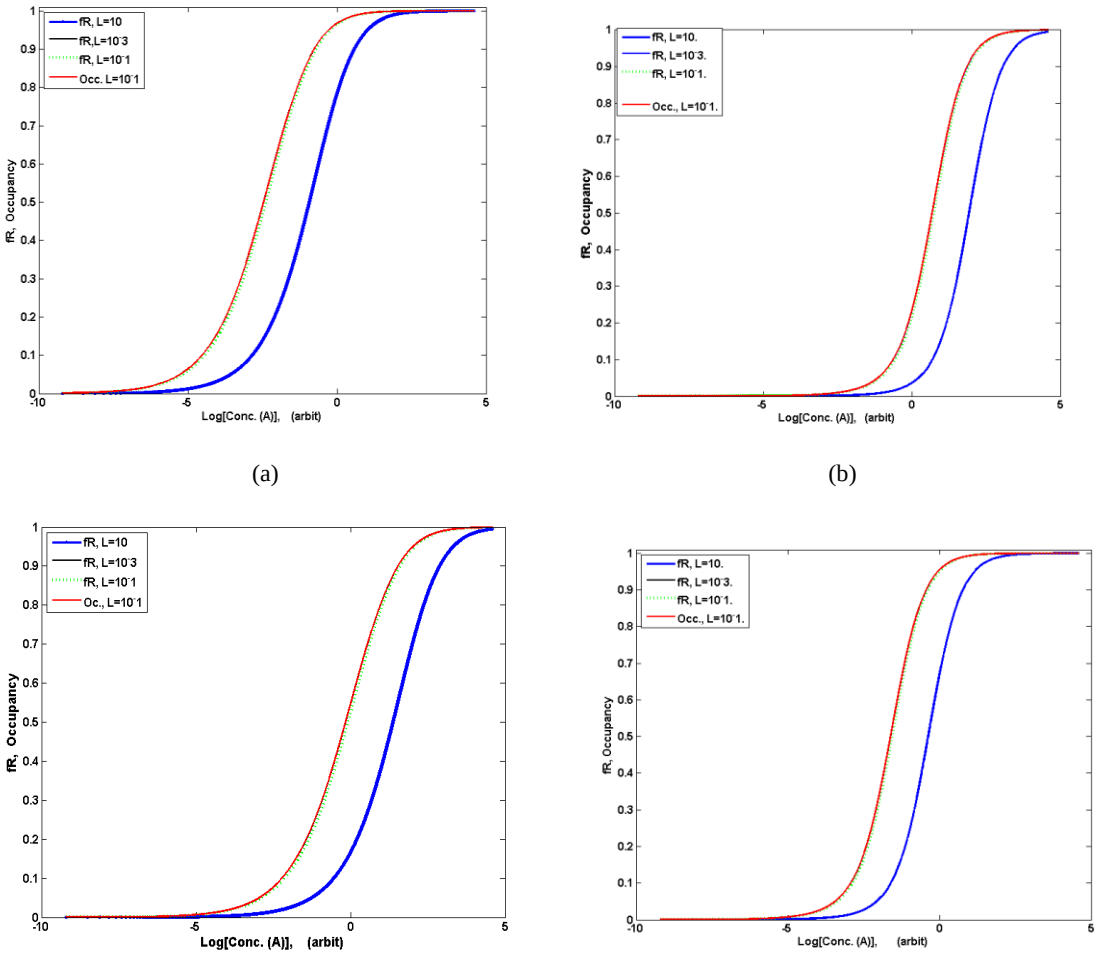


Figure 8: The plots show fraction of bound WASP complex verses ligand concentration.

In Fig.(8) above we vary L to show the stability of the equilibrium for the model. In (a) $K_R = C = 10^{-1}$, $K_T = 1$ whiles ranges $10^{-3} \le L \le 10$ in steps of 100.In all the plots above the black, green and blue curves correspond to an increase in L in the given steps respectively for equation (24). The red curve for equation (25), $K_{ss} = 10^{-1}$ coincide exactly with the black curve in our model. In Fig. (8b) desorption is increased by increasing the

equilibrium dissociation constant $K_R \frac{k_{-1}}{k_1} = 10$. The shift of the plot in Fig.8(b) to the right compared to the plotFig.8(a) is the effect of the equilibrium dissociation constant K_R. The plot in Fig.8(a) saturates faster and at lower concentration of the ligand compared to Fig.8(b) implying when the adsorption rate is less than the desorption rate binding is slower.

In the plots of Fig.. 8(c) and Fig. 8(d) $K_R = 1$whiles K_T take values 10 and 10^{-1} respectively. The analysis follows as described in the previous sections.

3. Discussion

Understanding the dynamics of WASP in eukaryotic cells is a major step in solving/curing many diseases including Buruli ulcer and Wiskott-Aldrich syndrome. In this study we analyzed the kinetics of enzyme-proteins binding and have shown that the two time scales that appear in solving the Henry Michaelis Menten equation are experimentally not measurable. An alternative is to measure the fraction of the various species in the reaction. The introduction of the intrinsic isomeric equilibrium constant L accounts for proteins that can exist in more than one conformational state. The ratio of equilibrium dissociation constant C, in the model, account for the affinity of one conformational state of the protein to a ligand over the other. The results show differences in active bound conformation of receptor complexes and bound receptor complexes. We find that in the absence of a ligand, the fraction of active WASP complex is controlled by the intrinsic isomeric equilibrium constant L whiles at saturating concentration of the ligand (Cdc42) it is controlled by the affinity constant C, and L.

4. Conclusion

The results show a rise in the fraction of active WASP complex as a result of its affinity for Cdc42 which confirms experimental reports in the literature. When the affinity of WASP for the ligand is high ($C < 1$) the stability of equilibrium is shifted to the left and vice versa (Figure 8). We have shown that in the absence of a ligand, the WASP protein is controlled by the isomeric equilibrium constant L. The fraction of active WASP complex exhibit autoinhibition in response to an increase in ligand concentration whiles the fraction of bound WASP complex exhibits saturation when the ligand concentration is increased. The model in this study can also predict existing models from Laidler and Hoare (1949), Haldane JBS. (1930) for L =0. Our results further augment the understanding of WASP autoinhibition in eukaryotic cell by its activator Cdc42.

References

Abdul-Manan, N., B. Aghazadeh, et al. (1999). Structure of Cdc42 in complex with the GTPase-binding domain of the 'Wiskott-Aldrich syndrome' protein. Nature 399(6734):379-383

Bindslev N. (2008) Drug-Acceptor interactions, DOI: 10.3402/bindslev.2008.5

Brian J. Galletta, Anders E. Carlsson, and John A. Cooper (2012) Molecular Analysis of Arp2/3 Complex Activation in Cells

Bruce A. Alexander J, Julian L. Martin R., Keith R. Peter W. (2008) Molecular Biology of the Cell, ISBN: 978-0-8153-4111-6.

Colquhoun D. (1998) Binding, gating, affinity and efficacy: the interpretation of structure-activity relationships for agonists and of the effects of mutating receptors Br J Pharmacol 125: 924_947

Derry, J. M., H. D. Ochs, et al. (1994). Isolation of a novel gene mutated in Wiskott- Aldrich syndrome. Cell 78(4): 635-644.

George E. B., HALDANE J.,B. S. (1925).L. A note on the kinetics of enzyme action.

Haldane JBS. (1930) Enzymes, 1st ed. London: Longmans, Green & Co,
Hall, A. (1998).Rho GTPases and the actin cytoskeleton. Science 279(5350): 509-514.

Hussain, N. K., S. Jenna, et al. (2001). Endocytic protein intersectin-l regulates actin assembly via Cdc42 and N-WASP. Nat Cell Biol 3(10): 927-932.

Jaffe, A. B. and A. Hall (2005). Rho GTPases: biochemistry and biology. Annu Review Cell. Dev Biol 21: 247-269

Kim, A. S., L. T. Kakalis, et al. (2000). Autoinhibition and activation mechanisms of the Wiskott-Aldrich syndrome protein. Nature 404(6774): 151-158.

Laidler KJ & Hoare JP. (1949) The molecular kinetics of the urea-urease system. I. The kinetic laws. J Am Chem Soc 71: 2699_2702, 1949

Lamarche, N., N. Tapon, et al. (1996). Rac and Cdc42 induce actin polymerization and G1 cell cycle progression independently of p65PAK and the JNK/SAPK MAP kinase cascade. Cell 87(3): 519-529.

Langmuir I. (1918) The adsorption of gases on plane surfaces of glass, mica and platinum. J Am Chem Soc 40: 13611403, 1918.

Laure G. M. Romain V. C. et. al (2013) Mycolactone activation of Wiskott-Aldrich syndrome proteins underpins Buruli ulcer formation JClinInvest.2013;123(4):1501–1512. doi:10.1172/JCI66576

Luke A H, Brad J N. (2013) Mechanism of synergistic activation of Arp2/3 complex by cortactin and N-WASP doi: 10.7554/eLife.00884

Matthias B, Wei Xu, and Michael K. R. (2001) Global disruption of the WASP Autoinhibited structure on Cdc42 binding. Ligand displacement as a novel method for monitoring Amide Hydrogen exchange

Miki, H., T. Sasaki, et al. (1998). "Induction of filopodium formation by a WASP-relatedactin-depolymerizing protein N-WASP." Nature 391(6662): 93-96.

Monod J, Changeux JP & Jacob F. (1963) Allosteric proteins and cellularcontrol systems. J Mol Biol 6: 306_329.

Miria Carmina Castiello et. Al (2013), Wiskott–Aldrich Syndrome protein deficiency perturbs the homeostasis of B-cell compartment in humans, doi: 10.1016

Murray J. D. (2001) Mathematical biology: an introduction

Munter, S., M. Way, et al. (2006). Signaling during pathogen infection. Sci STKE2006(335).

Panchal, S. C., D. A. Kaiser, et al. (2003). A conserved amphipathic helix inWASP/Scar proteins is essential for activation of Arp2/3 complex. Nat Struct Biol10(8): 591-598

Pollard, T. D. and J. A. Cooper (2009). Actin, a central player in cell shape and movement. Science 326(5957): 1208-1212.

Siripala, A. D. and M. D. Welch (2007). "SnapShot: actin regulators II." Cell 128(5): 1014

Stevens, J. M., E. E. Galyov, et al. (2006). Actin-dependent movement of bacterial pathogens. Nat Review Microbiol 4(2): 91-101.

Stewart, D. M., S. Treiber-Held, et al. (1996). Studies of the expression of the Wiskott- Aldrich syndrome protein. J Clin Invest 97(11): 2627-2634.