

Aggregation in Huntington's Disease: Insights through Modelling

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Abstract

Huntington's Disease (HD) is a late-onset, progressively degenerative brain disorder characterized by cell loss in the striatum and cortex. HD is caused by a polyglutamine (polyQ) expansion in the protein huntingtin (Htt). The mutant Htt is a substrate for caspases -2, -3 and -6. The cleavage of mutant Htt by caspase-2 has been suggested to underlie the selective neuronal death in HD. Once the mutant Htt is cleaved, a sticky and toxic fragment with the potential to form aggregates is released. The role of aggregation in the progression of HD has been extensively studied, yielding a plethora of ambivalent results. It has been shown that these are the diffuse, monomeric and oligomeric, forms of the mutant Htt fragment rather than the aggregates that are the major source of toxicity to the cells. We present here a mathematical model for aggregation in HD and discuss how it can relate to the selective neuronal death and the dependence of the disease onset on polyQ length. We describe the dynamical behavior of caspase-2, the release of monomeric forms of the mutant Htt fragment and the aggregation of these fragments through intermediate steps. Our model predicts that the concentration of toxic, intermediate oligomeric structures does not increase with increased caspase activity. We therefore suggest that the intermediate oligomeric forms of toxic Htt fragment do not account for selective and polyQ dependent neuronal death.

Keywords: huntingtin, exon1, caspase, aggregation, apoptosis, mathematical model

1 Introduction

Huntington's Disease (HD) is an inherited, progressively degenerative brain disorder that results in the loss of both mental faculties and physical control. At present there is no effective treatment for HD. In 1993 a search for a genetic basis of HD identified IT15 (interesting transcript 15) on chromosome 4 as the gene causing HD, and an expanded CAG repeat in the first exon of IT15 as the mutation underlying HD [13]. The CAG repeat is translated into a polyglutamine (polyQ) tract which is located in the N-terminal part of the large 350 kDa protein, named huntingtin (Htt). The elongated polyQ repeat interferes with the proper folding of the huge Htt and changes its interaction pattern with a number of other proteins, thus presumably affecting normal functions of Htt like clathrin-mediated endocytosis, neuronal transport processes, and protection from apoptosis. Since there are nine late-onset, progressive neurological disorders that are caused by polyQ expansion in otherwise unrelated proteins, it was natural to investigate biophysical properties induced by the prolonged glutamine tract itself. It was soon shown that polyQ tract causes the proteins to misfold and to form aggregates in groups of neurons specific for a given disease. Aggregation has been extensively studied *in vitro* and *in vivo*, in cell lines and in animal models.

Htt is expressed throughout the life, but the symptoms usually appear in an individual between 30 to 50 years of age and progress over a period of 15 to 20 years [9]. A number of researchers showed

that the most important cause for the variability in age of onset is the length of the polyQ repeat. The longer the repeat in Htt, the earlier and the more severe symptoms occur [2]. In HD patients with adult onset, mutant huntingtin (mut Htt) contains 36-55 glutamine repeats, and more than 60 in patients with juvenile onset. Htt is expressed in all cells so far tested [12]. Nevertheless, only specific areas of the brain are affected in HD. The most vulnerable cells are medium spiny neurons in the striatum, which are the first to die. Selective neural cell death is associated with choreic movements and dementia as clinical symptoms. As the disease progresses, regions of the cortex and basal ganglia start to show atrophy as well, leading to a 20% decrease in the weight of the entire brain [15].

In this article we address several questions about HD from a mathematical modelling perspective.

(1) How can we explain onset of the disease by threshold phenomena, (2) how can we reconcile the dual role of aggregates as both a marker for disease progression and as a protective mechanism, and (3) what is the source of increased toxicity in cells with longer polyQ repeats.

1.1 Road to Cell Death: Role of Caspase-2

Neuronal dysfunction underlies the clinical symptoms of HD, and the extent of cell death in striatum provides a basis for grading the severity of HD [15]. There is ample evidence that mitochondrial changes are important in the cell death decision in HD [6]. Activated caspase-2 causes the mitochondrial pores to open and cytochrome *c* to be released [8], that starts the cascade of events leading to cell death. Downstream of the first exon (exon1) of Htt are several consensus sequences recognized by caspase-3, caspase-2, and caspase-6 [16]. Therefore, all these caspases might be responsible for the Htt cleavage, which would release a short, sticky, and toxic N-terminal fragment. For our model, we considered only the role of caspase-2 for the following reasons: (1) It was shown that wild-type huntingtin upregulates transcription of brain-derived neurotrophic factor (BDNF), a pro-survival factor produced by cortical neurons that is necessary for the survival of striatal neurons in the brain. This beneficial activity of Htt is lost when the protein becomes mutated, resulting in decreased production of cortical BDNF [18]. Caspase-2 is a default executioner caspase as well as an initiator caspase in neuronal cells that have been deprived of nerve growth factor [14]. (2) Hermel and coworkers showed that Htt is cleaved *in vivo* at the caspase-2 consensus sequence, that caspase-2 immunoreactivity is enhanced in medium spiny neurons of the striatum in the YAC72 transgenic mouse model of HD when compared to controls, and that upregulation of caspase-2 correlates directly with decreased levels of BDNF in the cortex and striatum of the transgenic mice [4]. Their data support the involvement of caspase-2 in the selective neuronal cell death in the striatum and cortex in HD.

1.2 Mechanism of Aggregation and Toxicity

The late onset of the disease, strongly correlating with the polyQ repeat length [2], led to the conclusion that the aggregates are formed through a nucleation mechanism [7]. Nucleation is a sudden phase-transition phenomenon, in which, when a critical concentration is reached, the formation of oligomers has a higher probability of growing into aggregates than decomposing into monomers. Since nucleation is a slow and rate-limiting process, the time course of aggregation is sigmoidal with a lag time at the beginning (see, for example Figure 2). The growth rate of the aggregates increases with the number of glutamine repeats [11]. The process of aggregation involves the formation of at least three intermediates: monomers of Htt fragment, soluble oligomers and large insoluble aggregates. Of the three, aggregates have been the most extensively studied. Whether aggregates themselves are toxic, or whether they are a way a cell deals with an excess of undigested malfunctioning protein, has been a topic of intense discussion. Aggregates are defined by poor solubility in aqueous or detergents solvents, aberrant cellular localization and non-native secondary structure. Giant aggregates, or aggregates of aggregates are called inclusion bodies [5]. Recently, Arrasate and coworkers performed a survival analysis of individual cells expressing mut Htt. They showed that it is the amount of diffuse intracellular Htt that predicted whether and when cell death would occur. Their results support the

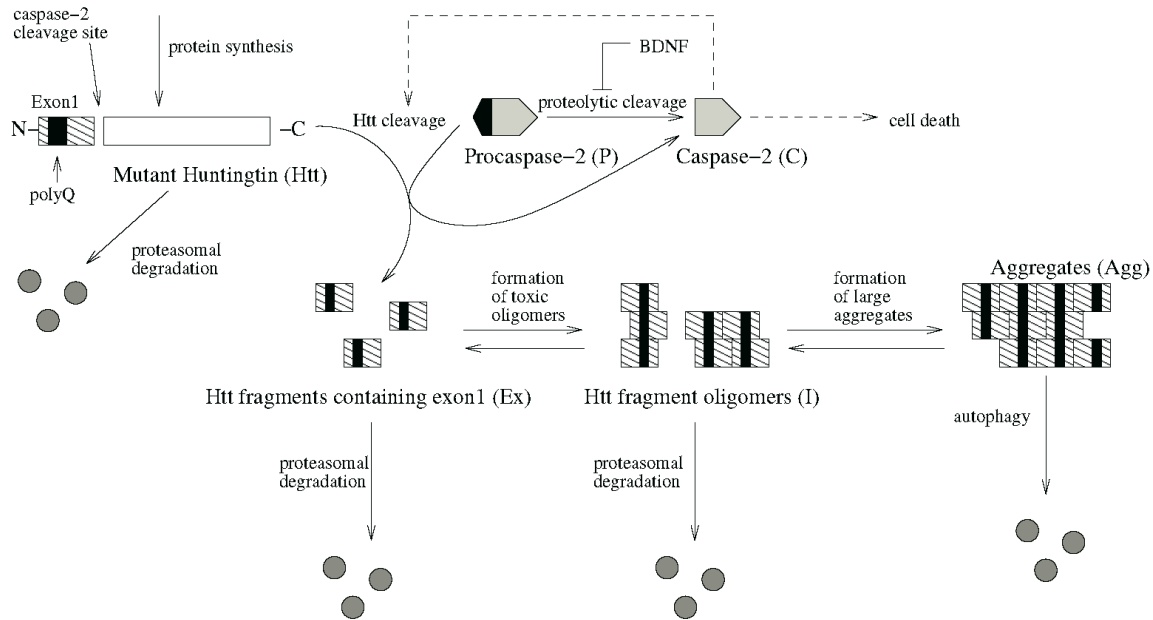


Figure 1: Model for caspase-2 mediated huntingtin aggregation. Mutation in Htt results in lower expression of BDNF, which leads to the activation of caspase-2. Mut Htt is cleaved by both procaspase-2 and caspase-2, and Htt fragment is released. Complex interactions of procaspase-2 and mut Htt not only release Htt fragment, but also initiate autocatalysis of procaspase-2 into caspase-2. Htt fragments aggregate through a succession of oligomeric structures. Htt, and monomers and oligomers of Htt fragments are degraded by the proteasome, while aggregates are removed by autophagy.

idea that inclusion bodies protect neurons by decreasing levels of toxic diffuse forms of mut Htt [1]. In the model presented in this paper we include only aggregates that are in the dynamical relation to the intermediate oligomeric structures.

2 Model for Aggregation in HD

The model for Htt aggregation is composed of 6 variables: full length mut Htt Htt , Htt fragment containing exon1 Ex , intermediate aggregates of Htt fragments or small oligomers I , large aggregates Agg , procaspase-2 P , and caspase-2 C (Figure 1). The full length mut Htt is cleaved by procaspase-2 which releases a short fragment containing exon1. Monomeric fragments start to aggregate through a number of intermediate oligomeric structures I . Both monomeric and oligomeric structures are substrates for proteasomal degradation. As the oligomeric structures get bigger they become more resistant to proteasomal degradation, thus the corresponding rates decrease (Table 1). The proteasomal machinery cannot digest big aggregates, so the cell removes them by autophagy. Mutation in Htt leads to a decrease in BDNF. This decrease is sensed by procaspase-2, which becomes active through proteolytic cleavage. Activation of caspase-2 initiates a cascade of apoptotic events leading to cell

Table 1: List of parameters.

Parameter	Value	Description
k_h	0.5	Htt synthesis rate
k_{dh}	1	Htt proteasomal degradation rate
k_{de}	0.1	Htt fragment proteasomal degradation rate
k_n	0.3	Rate of formation of oligomers
k_n^{-1}	0.1	Rate of release of monomers from oligomers
k_{dn}	0.05	Proteasomal degradation of oligomers
k_a	0.4	Rate of aggregation of oligomers
k_a^{-1}	0.05	Rate of release of oligomer from aggregates
k_{da}	0.1	Removal of aggregates by autophagy
k_p	0.2	Procaspase-2 synthesis rate
k_{dp}	0.05	Procaspase-2 degradation rate
k_{dc}	0.05	Caspase-2 degradation rate
k_{ce}	2.0	Maximal caspase-2 mediated cleavage rate
k_{pe}	0.1	Maximal procaspase-2 mediated cleavage rate
k_{pr}	0.05	Proteolytic cleavage rate of procaspase-2
K_i	1.0	Saturation constants of cleavage rates ($i = 1, \dots, 3$)

death. These mechanisms can be represented by the following equations:

$$\frac{dHtt}{dt} = k_h - r_{ce} - r_{pe} - k_{dh}Htt, \quad (1)$$

$$\frac{dEx}{dt} = r_{ce} + r_{pe} - k_n Ex I_n + k_n^{-1} I - k_{de} Ex, \quad (2)$$

$$\frac{dI}{dt} = k_n Ex I - k_a I Agg + k_a^{-1} Agg - [k_n^{-1} + k_{dn}] I, \quad (3)$$

$$\frac{dAgg}{dt} = k_a I Agg - [k_a^{-1} + k_{da}] Agg, \quad (4)$$

$$\frac{dP}{dt} = k_p - 2(r_{pe} + r_{pr}) - k_{dp} P, \quad (5)$$

$$\frac{dC}{dt} = 2(r_{pe} + r_{pr}) - k_{dc} C, \quad (6)$$

where the nonlinear rates r are

$$r_{ce} = k_{ce} \frac{Htt C}{1 + K_1 P + K_2 C + K_3^2 P^2}, \quad (7)$$

$$r_{pe} = k_{pe} \frac{Htt P^2}{1 + K_1 P + K_2 C + K_3^2 P^2}, \quad (8)$$

$$r_{pr} = k_{pr} P^2. \quad (9)$$

The rates are derived from law of mass-action, for which we assume that the rates of formation of the complex between Htt and procaspase-2, and Htt and caspase-2 are fast enough for intermediate reactions to reach their equilibrium [3]. This allows us to derive the nonlinear rates r . The term r_{ce} is the rate of Htt cleavage mediated by active caspase-2. The term r_{pe} is the rate of a complex reaction in which, through interaction with one another, both mut Htt and procaspase-2 get cleaved. Finally, r_{pr} is the proteolytic activation rate of procaspase-2. The quadratic terms P^2 and the factors 2 in the above equations result from the formation of procaspase-2 homodimers. The parameters are described in Table 1. Since quantitative estimations of concentrations and time scales varies greatly according to experimental settings, we chose to assign arbitrary time and concentration units to the parameters. The parameter values were chosen according to qualitative estimates of aggregation kinetics. The results shown here yield for a wide range of parameter values as long as Htt synthesis rate k_h is sufficiently large.

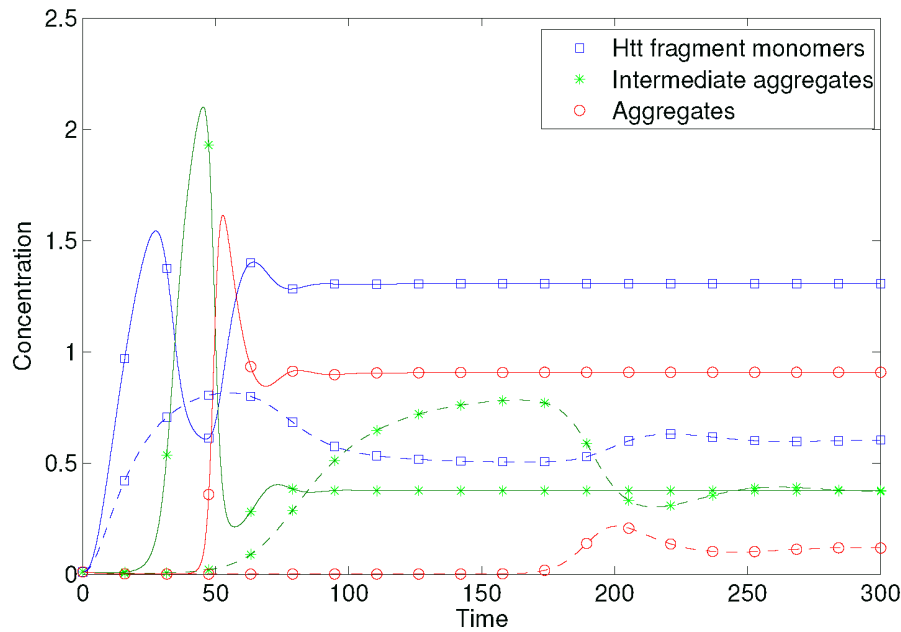


Figure 2: Evolution of Ex (\square), I ($*$) and Agg (\circ). Two time courses are shown, one for low (*dashed*) and one for high (*solid*) caspase-2 proteolytic cleavage rate ($k_{pr} = 10^{-4}$ and 0.1). Notice the overshoot of the Htt fragment monomer and oligomer concentration before aggregation starts. With higher active caspase-2 concentration, only concentrations of aggregates and monomers increased. Intermediate oligomers stabilize to the same level as with a lower caspase-2 activation rate. Parameters as in Table 1, except for k_{pr} .

3 Results

3.1 Behaviour of the Full Model

The simulation of the model consisting of equations (1–6) shows that an increase in caspase activity mainly affects the aggregate and monomer concentrations (Figure 2). At time $t = 0$, all concentrations were set to low values (0.01). Time ran from $t = 0$ to $t = 400$. After a transient peak, monomer and oligomer concentrations reach a steady state as aggregates start to form. Although with a higher Htt cleavage rate, the aggregate and monomer concentrations increase, the steady state concentration of intermediate aggregates does not seem to be influenced. Aggregates act as a buffer, binding the newly formed oligomers, and thus keeping their concentration constant. To further investigate the effect of caspase-2 mediated cleavage on aggregation kinetics, we considered a reduced model that can be treated analytically.

3.2 Kinetics of a Reduced Model

To explore the aggregation kinetics, we assumed that caspase activity is constant by setting P and C to constant values. This allows an analytical treatment of the reduced system consisting of equations (1–4). Fixing the caspase activity, we could re-express the nonlinear rates r_{ce} and r_{pe} as $r_{ce} + r_{pe} = Htt \cdot A$, with A as the caspase-mediated Htt cleavage rate,

$$A = \frac{k_{ce}C + k_{pe}P^2}{1 + K_1P + K_2C + K_3^2P^2}. \quad (10)$$

The caspase-mediated cleavage rate A will be the main bifurcation parameter in the reduced model. We show basic properties of aggregation that remain valid when the full system is considered.

Table 2: steady states of equations (1-4).

	STST1	STST2	STST3
Htt	$\frac{k_h}{A + k_{dh}}$	$\frac{k_h}{A + k_{dh}}$	$\frac{k_h}{A + k_{dh}}$
Ex_i	$\frac{AHtt}{k_{de}}$	$\frac{k_{dn} + k_n^{-1}}{k_n}$	$\frac{AHtt + k_n^{-1}I_3}{k_n I_3 + k_{de}}$
I_i	0	$\frac{AHtt - k_{de}Ex_2}{k_{dn}}$	$\frac{k_a^{-1} + k_{da}}{k_a}$
Agg_i	0	0	$\frac{I_3}{k_{da}} [k_n Ex_3 - (k_{dn} + k_n^{-1})]$

For any set of kinetic parameters, three steady states always exist, of which only one can be stable (Figure 3). The three steady states are listed in Table 2. The subscripts i in Ex_i , I_i and Agg_i refer to the i th steady state (STST i , $i = 1, \dots, 3$). For low caspase activity, only monomers are present, in a concentration too small for oligomeric structures to be stable, thus STST1 prevails. As the caspase activity increases the system goes to STST2, where oligomers become stable but aggregates are still unstable. A further increase in caspase activity brings the system to STST3 where all three entities, monomers, oligomers, and aggregates, are present. Two threshold levels of caspase activity A_1 and A_2 define transcritical bifurcation points, where an exchange in stability from one STST to another occurs. The transition from one STST to the other must be continuous, and only changes from STST1 to STST2 and STST2 to STST3 are possible. This requirement excludes bistable behaviour of the subsystem. The two bifurcation points A_1 and A_2 are defined as follows:

$$A_1 = \frac{k_{dh}a}{k_n k_h - a}, \quad (11)$$

$$A_2 = \frac{k_{dh}(a + b)}{k_n k_h - (a + b)}, \quad (12)$$

$$a = k_{de}(k_{dn} + k_n^{-1}), \quad (13)$$

$$b = k_{dn}k_n \frac{k_a^{-1} + k_{da}}{k_a}. \quad (14)$$

For high cleavage rates ($A > A_2$), the formation of aggregates is responsible for most of the change in concentration; monomers concentration increases slowly and the concentration of intermediate oligomers stays constant. Aggregates act as a buffer to limit the increase in concentration of oligomers of Htt fragments. Moreover, the steady state value of oligomers (I_3) depends only on the rates related to the formation of large aggregates k_a , k_a^{-1} and k_{da} , and not on particular levels of monomers Ex or aggregates Agg . A simulation with the reduced model was performed with varying caspase activity (Figure 4). The cleavage rate A was changed from $A = 0.05$ for $t = 0$ to 80, $A = 0.15$ from $t = 80$ to 160, $A = 0.5$ from $t = 160$ to 320 and $A = 2$ from $t = 320$ to 400. After a lag phase, aggregates start forming around $t = 250$ and the concentration of intermediate aggregates decreases considerably to reach a steady state level ($I_3 = 0.375$). At $t = 320$, after a four-fold increase of A , the level of intermediate aggregates rapidly readjusts to its steady state level. The concentrations of monomers and aggregates correlate with the caspase activity.

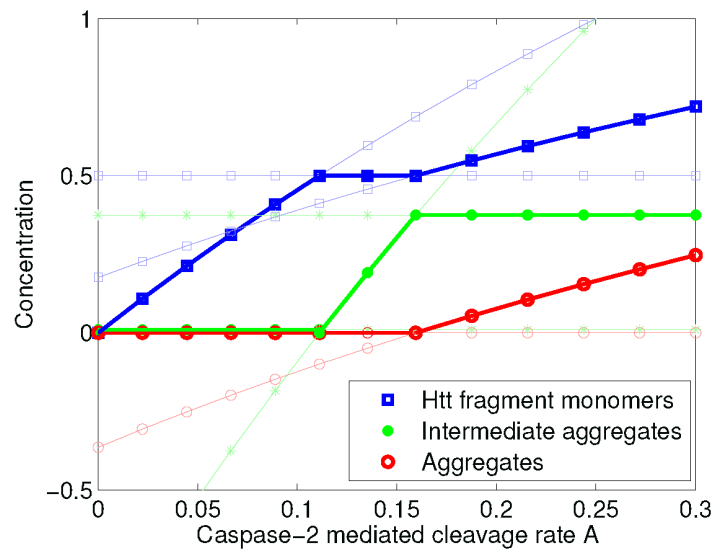


Figure 3: Bifurcation diagram of aggregation. The steady states for Ex (\square), I ($*$) and Agg (\circ) are plotted as a function of cleavage rate A . The bifurcation analysis shows that as the cleavage rate by caspase-2, A , varies, the system goes successively through three steady states. For low caspase activity, only monomers are present, in a concentration too small for oligomeric structures to be stable, and STST1 prevails. When A is larger than the threshold $A_1 = 0.11$, stable small oligomers form, yet large aggregates are still unstable, and the system is at STST2. If A is increased above the second threshold $A_2 = 0.16$, large aggregates also start to form, as defined by STST3. The two threshold levels A_1 and A_2 define transcritical bifurcation points, where an unstable steady state (*solid lines*) exchanges stability with a stable steady state (*bold lines*). Notice that for larger A , Ex_3 increases more slowly than Ex_1 . More interestingly, the steady state I_3 is constant, that is, I_3 is independent of the caspase activity.

4 Discussion

In this paper we present a mathematical model for aggregation in Huntington's disease in which the process is activated by procaspase-2 and caspase-2. The model consists of six differential equations with mutant huntingtin, monomers, oligomers, aggregates, procaspase-2 and caspase-2 as variables. It reproduces qualitative behaviour observed in the experiments, namely: (1) activation of caspase-2 by the lack of BDNF and by the interaction with mutant huntingtin, (2) existence of a lag phase during the process of aggregation, and (3) no strict correlation between an increase in the level of aggregates and an increase in cell death.

We have shown that the onset of aggregation is well explained by threshold phenomena. Upon the initiation of the procaspase-2 and caspase-2 mediated cleavage of mut Htt, two transcritical bifurcations lead to aggregation. Both environmental changes and toxic effects of mut Htt in the cell can further activate caspase-2, which will increase the level of monomers. On the other hand, these changes do not increase the level of oligomers in the cells because the oligomers get recruited by large aggregates. The level of oligomers is determined only by the rates related to aggregates: k_a , k_a^{-1} , and k_{da} , and not by the concentration of monomers (and, therefore, not by caspase-2 activity). We have shown that once the activity of caspase-2 is above a certain threshold, all three entities are present in the system (STST3), and a further increase of the caspase-2 activity increases only the levels of monomers and aggregates. It is likely that at the time a person is diagnosed with HD, affected neuronal cells are already in the STST3. The model suggests that as the disease progresses there is no increase in the concentration of oligomeric forms and that, therefore, they can not account for the increased toxicity.

So far we have discussed how the change in one parameter, caspase-2 activity, influences the

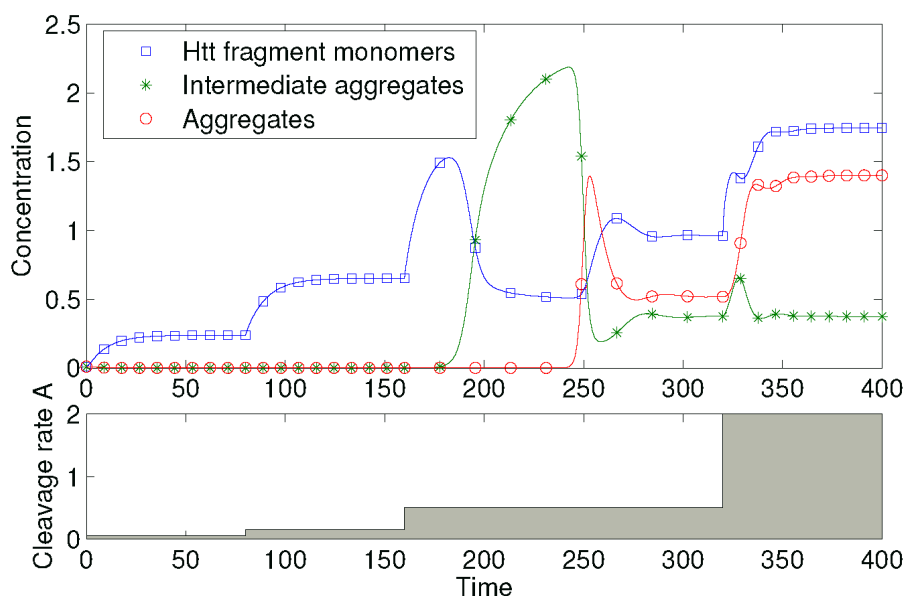


Figure 4: In the upper panel, time courses of Ex (\square), I ($*$), and Agg (\circ) are shown. Caspase-2 activity increase is simulated and indicated by the gray shading (*lower panel*). When aggregation starts around $t = 250$, the level of I decreases to reach a steady state that remains constant even for further increase of caspase-2 activity, monomer concentration Ex , and aggregates Agg . The values of caspase-mediated cleavage A are successively 0.05, 0.15, 0.5, and 2.

formation of toxic fragments. Indeed, this has been the major topic of this paper. We would now like to briefly consider the changes that might be brought to the system with the increase in the repeat length. The likelihood of procaspase-2 mediated cleavage of a polyQ containing protein increases with the repeat length, leading to increased Htt fragment release. The aggregation rates rise when the polyQ length is increased, and we would expect monomers to be recruited faster to oligomers and oligomers to be recruited faster to aggregates. Additionally, all degradation rates would decrease since the structures would become more resistant to removal by the proteasome. Our model suggests that in this case again, the concentration of monomers and aggregates would go up, but the concentration of intermediate oligomers would drop. The steady state value of intermediate aggregates in STST3 is $(k_a^{-1} + k_{da})/k_a$. With longer polyQ length, the numerator would tend to decrease and the denominator to increase. This unexpected feature of aggregation kinetics gives a cue for the source of additional toxicity. It would come from either monomers or from aggregates. Recent data suggests that aggregates have a protective role by converting toxic forms of Htt fragments into less toxic ones. In this case, our results suggest that Htt fragment monomeric form might be responsible for elevated toxicity and underlie selective and polyQ dependent neuronal

Much effort has been involved in finding substances that interfere with aggregation, either by direct inhibition with Htt protein as a target, or by indirect inhibition by, for instance, caspase inhibitors. In various cell and mouse models caspase inhibitors have been shown to reduce toxicity and aggregate formation [17]. Interference with the later steps, i.e., oligomerization and aggregation, has been shown to be beneficial for the cell as well [10]. Taking into account the complexity of our dynamical system, mathematical modelling can be helpful in reconciling apparently contradictory results. In a future paper we will investigate in detail the influence of intervention at various steps in our model on the overall toxicity.

Acknowledgments

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