Hypothesis

Autocatalytic processes in cooperative mechanisms of prion diseases

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Abstract According to the leading theory, the agent responsible for prion diseases would be the conformational isomer PrP^{Sc} of a cellular protein PrP^{C}, the pathogenic form PrP^{Sc} multiplying by converting the normal protein into a likeness of itself. The pathogenic isoform could catalyze the conformational transition so that the process, taken as a whole, is autocatalytic. However, in this simple but atypic model, unrealistic values of rate parameters are needed in order to account for the kinetics of the propagation of prion diseases. In this paper, I show that these limits can be overcome by assuming that catalysis proceeds through a multimeric assembly of the pathogenic isoform of the prion protein. Such a structure would indeed be able to provide cooperativity both at the assembly and conformational change levels, strongly reinforcing the autocatalytic character of the activated process. Moreover, such a property is a prerequisite to endow the metabolic system with dynamic bistability. Together with a good agreement regarding experimental data, this analysis is closely akin to Griffith's original idea concerning the thermodynamic conditions required for autocatalyzed modifications of any protein.

Key words: Prion disease; Cooperativity; Autocatalysis; Subunit interaction; Allostery

1. Introduction

Compelling evidence [1–4] strongly suggests that a post-translational structural alteration in a glycoprotein PrP^{C} (the normal, cellular isoform of the so-called prion protein) is responsible for pathogenesis of transmissible spongiform encephalopathies (or prion diseases). No chemical difference has been detected so far between PrP^{C} and PrP^{Sc}, the modified, pathogenic form of the protein. On the other hand, the two forms differ in their physicochemical properties and in their secondary structure [5–7]. Moreover, Bessen et al. [8] took advantage that two hamster-adapted mink strains give rise to two distinguishable PrP^{Sc} molecular species to show that, in a cell-free system, PrP^{Sc} from the two strains can convert the same PrP^{C} protein into two distinct sets of products that have the same physicochemical properties than those of natural PrP^{Sc} molecules associated with the two strains. These data support the idea that PrP^{C} and PrP^{Sc} are conformational isomers and that the pathogen isoform PrP^{Sc} may impose its conformation upon the native protein PrP^{C}.

Whereas Griffith [9] first suggested that there are no thermodynamic reasons preventing the self-replication of proteins, Prusiner's group provided much experimental evidence in favor of the 'protein only' hypothesis. Moreover, Prusiner first proposed a molecular mechanism for an autocatalytic turnover of the host protein into its pathologic isoform [1]. In the corresponding model, the constitutive PrP^{C} protein would be unfolded to some extent and subsequently refolded under the influence of PrP^{Sc} molecules. As a derivative, Liautard suggested that prions could be misfolded molecular chaperones [10]. This globally autocatalytic process requires the presence of preexisting PrP^{Sc} which is supposed to be formed very slowly by spontaneous conversion. Inherited prion diseases are linked to one of a number of mutations in the *PrP* gene which are assumed to increase the frequency of the spontaneous conversion of PrP^{C} into PrP^{Sc}.

However, Eigen recently argued [11] that quite unrealistic values of rate parameters are needed in order to allow such a process to occur. The mechanism has to navigate between an extremely low value of the first-order rate constant (k_s) of spontaneous conversion (otherwise pathogenic form will grow spontaneously even without infection) and a rather high value of the catalytic constant (k_{cat}) of the activated process of conformational change (otherwise the mechanism could never become effective, even in the presence of infection). According to Eigen, the catalytic enhancement (being expressed by the ratio k_{cat}/k_s) has to be larger than 10^{15}. We are not aware of any non-cooperative enzymatic turnover which realizes such a rate enhancement.

Alternatively, I recently suggested [12,13] that the infective mechanism of prion diseases does not necessarily need to be viewed as an explosive mechanism (i.e. a non-equilibrium mechanism in which infection only accelerates a process which is otherwise too slow to become fatal during the normal lifetime of the organism). Assuming only a positive feedback loop in the catalyzed conversion between PrP^{C} and PrP^{Sc} isoforms of the protein, we can show that the metabolic system which involves the prion protein possesses bistability properties. Hence, infection would correspond to a switch between two alternative stable steady-states. Convergent and similar theoretical analysis was performed simultaneously by Kacser and Small [14].

Hence, a possible cooperative mechanism of conversion between PrP^{C} and PrP^{Sc} would not only be much more realistic with regard to catalytic prerequisites but be also of a great interest for the understanding of the dynamic of prion invasion. Experimental data [15] supports the contention that PrP^{Sc} could act as a multimeric assembly in the catalytic process. In this paper, I derive the thermodynamic requirements allowing a cooperative conformational change of prion protein to occur and show how such a process would enhance the autocatalytic effect compared to the simple mechanism of Prusiner.

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2. Results

Whatever the molecular mechanism of the catalyzed conversion between PrP^C and PrP^{Sc} isoforms of the prion protein, the product of the reaction (the PrP^{Sc} isoform) is also part of the catalyst (or the catalyst itself in the mechanism of Prusiner). This characteristic provides an autocatalytic enhancement of the conversion rate. However, in the unimolecular mechanism of catalysis, amplification is linear because the velocity increases proportionally to the concentration of the product of the reaction (without any modification of the rate constant). Although conformational change looks like some aspects of the induced-fit hypothesis (which plays an important role in the cooperative properties of allosteric enzymes), no cooperative effect may be expected in such a model in which the catalyst is a monomer. A quite different situation prevails if we supposed that any oligomeric form of PrP^{Sc} can act as a catalyst.

The concepts of enzyme cooperativity and allostery have been formulated 30 years ago [16–18]. However, the mechanism of conversion between PrP^C and PrP^{Sc} isoforms of the prion protein presents unusual and interesting characteristics, since the concentration of the catalyst increases as the reaction progresses. Fig. 1 shows the global scheme corresponding to the case in which both monomeric and dimeric forms of PrP^{Sc} can convert the normal isoform PrP^C of the prion protein. Two possible pathways for achieving conformational changes may be considered: (1) an initial conversion of the first PrP^C subunit bound to the PrP^{Sc} isoform followed by the binding of the second PrP^C subunit to the changed PrP^{Sc}–PrP^{Sc} quaternary structure; (2) an initial binding of the two PrP^C subunits to the PrP^{Sc} isoform followed by a sequential conformational change. Thermodynamic considerations require that any pathway must give the same over-all equilibrium constant. Hence, without assuming that the corresponding direction is dominant, we shall analyse pathway 2 with the aim to clearly set apart binding steps from conformational transitions.

2.1. Cooperativity at the assembly stage

In a classical allosteric system in which the ligand is a small molecule, intrinsic binding steps are never cooperative by themselves [16–20]. The situation may be quite different for the prion protein since the size of the ligand (PrP^C) compares with that of the catalyst (PrP^{Sc} or PrP^{Sc}–PrP^{Sc}). The over-all assembly stage corresponds to two consecutive binding processes having distinct microscopic equilibrium constants K_1 (corresponding to $\Delta G1_{ass}$) and K_2 (corresponding to $\Delta G2_{ass}$). When $K_1 = K_2$, the scaled fractional saturation function Y of the catalyst PrP^{Sc} by PrP^C corresponds to a Michaelian (i.e. non-cooperative) binding isotherm. Conversely, when $K_1 < K_2$ (defined as association constants), PrP^C binding is cooperative, i.e. the binding of PrP^C to free PrP^{Sc} facilitates further binding of PrP^C to the PrP^{Sc}–PrP^C complex.

At the molecular level, cooperativity of the over-all binding process depends upon the stability of the subunits ideally isolated relative to the aggregated states, assuming that this process does not involve any conformational change [21].

2.2. Cooperativity of conformational transitions

Considering the associated heterotrimer PrP^{Sc}–$(PrP^C)_2$ in Fig. 1, the problem is now: in what ways conformational change in one PrP^C subunit can affect stability and/or conformation of neighboring subunits? Such a question directly pertains to the allosteric concept. The answer is quite simple if we assume that the region enclosing the complementary bonding sets of two monomers through which they are linked (i.e. the domain of bonding) is made up of two different binding sets (i.e. the associations between subunits are heterologous). On the other hand, if we suppose that the domain of bonding involves two identical bonding sets (isologous associations), infinite helical structure would be expected [22]. We shall define the following ideal energy contributions:

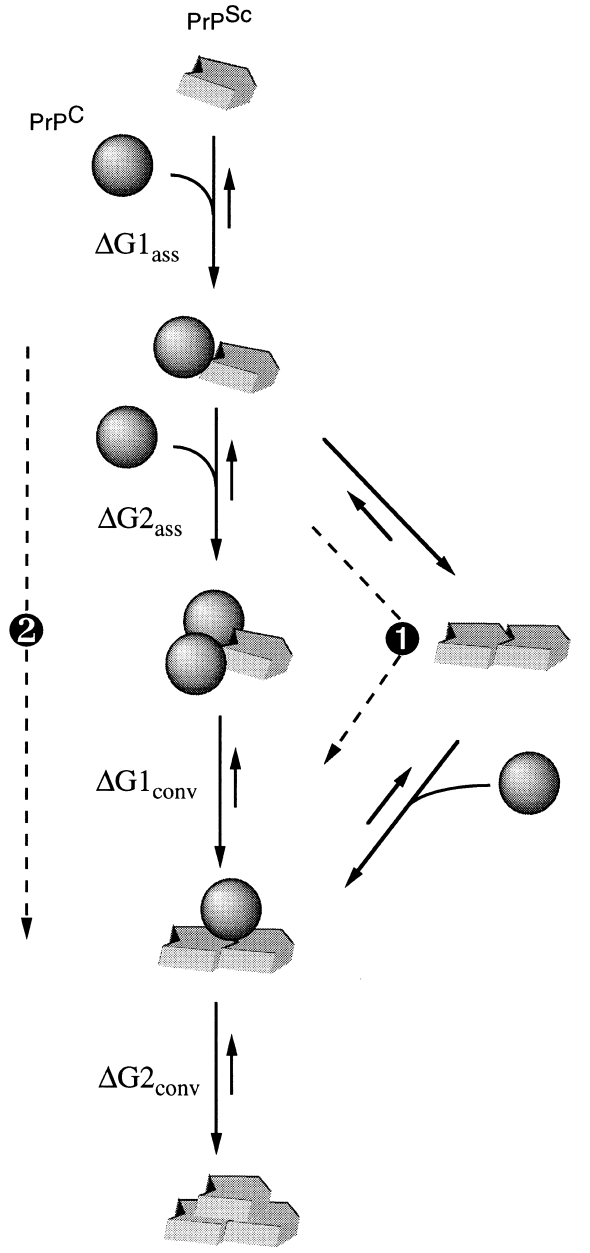

Fig. 1. Uni- and bimolecular catalytic processes involving the PrP^{Sc} isoform of the prion protein. The binary complex PrP^C–PrP^{Sc} can evolve along two alternative pathways (in addition to the reverse dissociation): either isomerization of PrP^C subunit into PrP^{Sc} isoform and subsequent binding of the second PrP^C molecule (pathway 1) or firstly binding of the second PrP^C subunit followed by the first conversion step of PrP^C into PrP^{Sc} (pathway 2). Both pathways are thermodynamically equivalent.

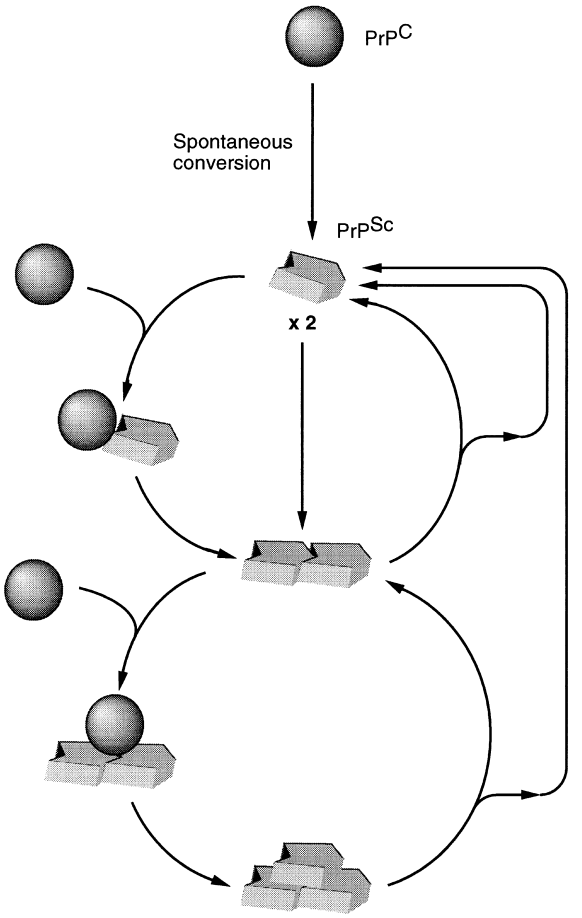

Fig. 2. Reaction cycles for the conformational transition between normal and pathogenic forms of the prion protein catalyzed by the monomeric and dimeric isoform PrP^{Sc}.

- ΔG_t the energy difference between free (i.e. dissociated) PrP^{Sc} and PrP^C subunits.
- ΔG_{CC} (*rs.* ΔG_{ScSc}) the relative free energy expressing the strength of interactions between two subunits in the same PrP^C (*rs.* PrP^{Sc}) conformation.
- ΔG_{CSc} the relative free energy expressing the strength of interactions between one subunit in the PrP^C conformation and another subunit in the PrP^{Sc} conformation. (Strictly speaking, ΔG_{CSc} expresses the mean free energy between the bonding sets *a* of a PrP^{Sc} monomer and *b* of a PrP^C monomer on the one hand and the bonding sets *b* of a PrP^C monomer and *a* of a Pr^{Sc} monomer on the other hand).

Hence, each of the global free energy change $\Delta G1_{conv}$ and $\Delta G2_{conv}$ in Fig. 1 may be split into the following contributions:

$$\Delta G1_{conv} = \Delta G_t + \Delta G_{ScSc} - \Delta G_{CC}$$
$$\Delta G2_{conv} = \Delta G_t + 2\Delta G_{ScSc} - 2\Delta G_{CSc}$$

Positive cooperativity will be observed in the global process of conformational change if and only if $\Delta G2_{conv} < \Delta G1_{conv}$, i.e.:

$$\Delta G_{CSc} > \frac{\Delta G_{CC} + \Delta G_{ScSc}}{2}$$

Thus, positive cooperativity in the conformational process will be observed if subunit interactions between PrP^C and PrP^{Sc} monomers are destabilizing compared to subunit interactions between monomers in the same conformation.

2.3. *Influence of cooperative effects on the rate of conformational changes*

Fig. 2 presents the general kinetic scheme corresponding to a mechanism in which the dimeric and trimeric complexes are active in the conversion process. In order to identify the key influences which can govern prion propagation, we shall isolate three limiting cases (Fig. 3) from this generic scheme. The model of Fig. 3A corresponds to the Prusiner's mechanism of unimolecular catalysis. It will be used as a reference. Com-

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Fig. 3. Comparison between the catalytic efficiency obtained in three particular models derived from the generic scheme of Fig. 2. A: Linear autocatalytic model of Prusiner. Compared to Fig. 3, dissociation of the complex $(PrP^{Sc})_2$ is supposed to occur faster than the putative binding of normal free subunit PrP^C to form a ternary complex. Moreover, uncatalyzed formation of homodimer PrP^{Sc}–PrP^{Sc} is neglected. B: Cooperative autocatalytic scheme with preferential interactions between PrP^C and PrP^{Sc} isoforms. In theses conditions, direct interactions between two PrP^{Sc} free subunits can be neglected compared to the interactions occurring between PrP^C and PrP^{Sc} isoforms. C: Cooperative autocatalytic scheme with preferential interactions between PrP^{Sc} isoforms. In this model, the opposite assumption concerning preferential interactions is considered: PrP^{Sc} is supposed to interact more easily with PrP^{Sc} rather than with PrP^C. Hence, except at the very beginning of the reaction, catalysis essentially acts through the dimeric form PrP^{Sc}–PrP^{Sc} of the catalyst. In the numerical simulations, arbitrary units of time [*t*] and concentration [*c*] were considered. Hence, we can demonstrate the nature of the system independently of the quantitative values of the parameters (yet unknown). Real units that would be given to the rate constants would fix absolute values on the graphical axes. In the three models, according to the classical hypothesis of rapid equilibrium in enzyme kinetics, association and dissociation processes are assumed to be fast compared to conversions ($k_{d0} = k_{d1} = k_{d2} = 1000\ [t]^{-1}$ and $k_{a2} = 1000\ [c]^{-1}[t]^{-1}$). Hence, global catalyzed steps:

$$(PrP^{Sc})_i(PrP^C)_j + PrP^C \rightarrow (PrP^{Sc})_{i+1}(PrP^C)_j$$

may be considered as Michaelian processes with an over-all rate *v* such as:

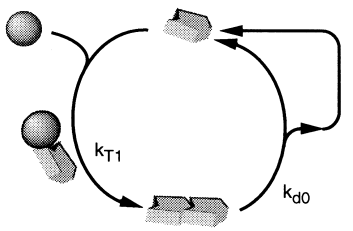
$$v = \frac{k_{cat}[PrP^C]}{K_M + [PrP^C]}[(PrP^{Sc})_i(PrP^C)_j]$$

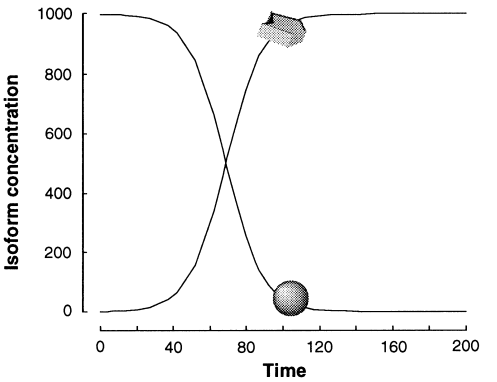
Finally, since the concentration of the prion protein probably never exceeds several hundred nanomolar, we supposed that $K_M \gg [PrP^C]$ (this assumption allows us to reduce the number of parameters, although the behaviour of the models does not depend on its validity). In these conditions, the global catalyzed steps may be considered as second-order processes with a rate constant $k_T = k_{cat}/K_M$. As a result of cooperativity, dimeric catalysis is assumed to be 100-fold more efficient than monomeric catalysis ($k_{T2} = 100\ k_{T1}$ with $k_{T1} = 10^{-4}\ [c]^{-1}[t]^{-1}$). Each simulation was performed by numerical integration of the differential equations governing the time evolution of the corresponding system, with initial conditions such as: $[PrP^C]_0 = 1000\ [c]$, $[PrP^{Sc}]_0 = 1\ [c]$ and the initial concentrations of other complexes equal zero.

pared to the general scheme of Fig. 2, the affinity of the complex $(PrP^{Sc})_2$ for free PrP^C subunits is supposed to be very low. Moreover, PrP^{Sc} is supposed to bind much more easily to PrP^C rather than to PrP^{Sc}. The second model (Fig. 3B) assumes that free PrP^{Sc} preferentially interacts with PrP^C subunits. In the third model (Fig. 3C), the opposite hypothesis is considered: free PrP^{Sc} subunits preferentially bind to PrP^{Sc} to constitute a catalytic homodimer.

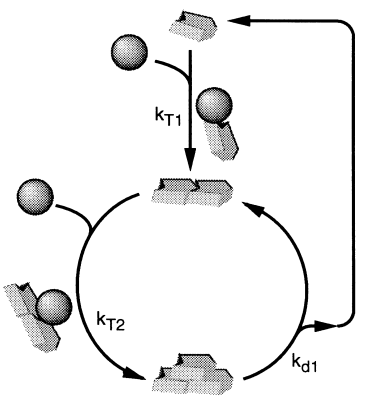
To characterize the catalytic efficiency in each of these schemes, we shall consider, in all numerical simulations, that a very low fraction of pathogenic form PrP^{Sc} (1/1000 of native protein PrP^C) is initially present in the medium, as a result of the spontaneous conversion. Assuming usual hypotheses of enzyme kinetics (see legends of Fig. 3), the rate of each catalyzed step (i) may be treated as a second-order rate law in which the rate constant $k_{T(i)}$ equals $k_{cat(i)}/K_{M(i)}$. The crucial point of this analysis is as follows: what ratio between k_{T1} (catalysis by the monomer PrP^{Sc}) and k_{T2} (catalysis by the

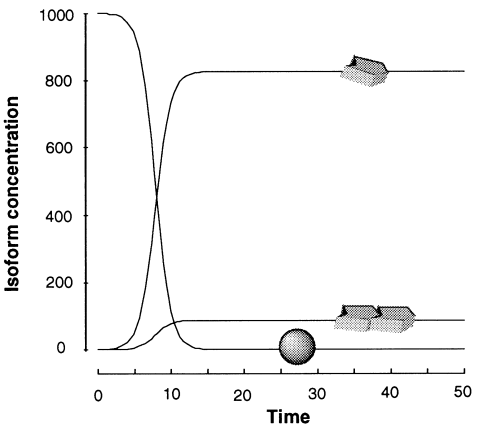
A. Linear autocatalysis (Prusiner)



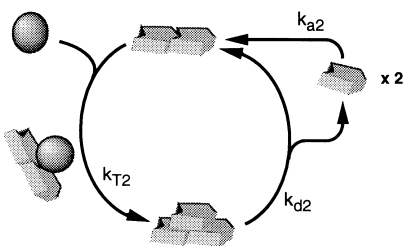


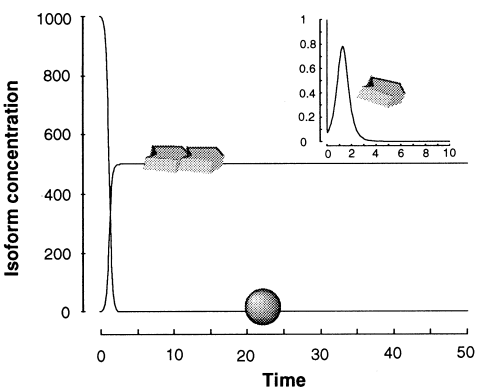
B. Cooperative autocatalysis with PrP^C-PrP^{Sc} preferential interactions





C. Cooperative autocatalysis with PrP^{Sc}-PrP^{Sc} preferential interactions





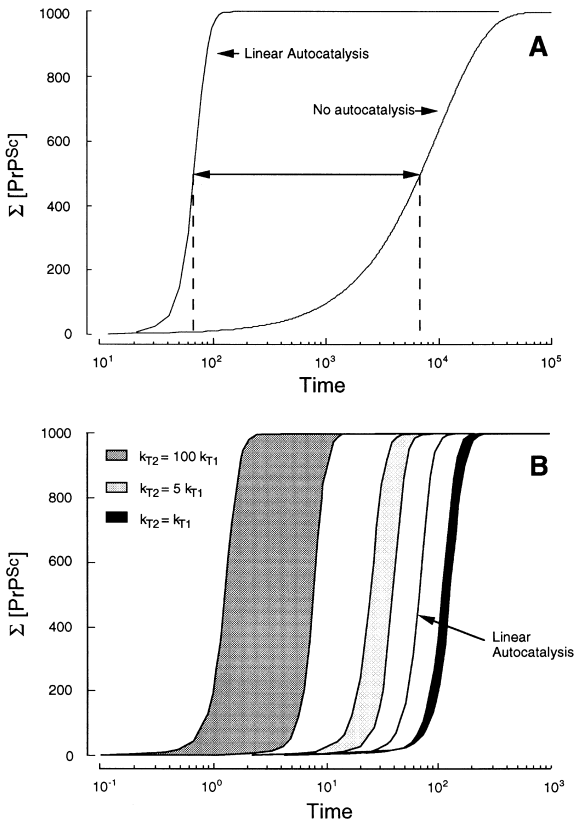


Fig. 4. Relative importance of the cooperative effect compared to that of linear autocatalysis. Starting from identical values of parameters and initial concentrations for all the simulations, the relative importance of each of these factors may be quantitatively assessed from the measure of half-reaction times. Except for k_{T2}, all other values were those of Fig. 3. A: Linear autocatalysis effect. Kinetics of conformation changes are compared for a simple exponential process (which corresponds to the case where the product of the reaction does not behave as a catalyst) and the Prusiner's mechanism of linear autocatalysis (Fig. 3A). B: Cooperative effect. In each of the models of Fig. 3B,C, three distinct simulations have been performed, with different values of the rate constant k_{T2} (which expresses the efficiency of the dimeric catalysis). For a given k_{T2} value, the interval between the curves corresponding to the two models has been drawn in a grey scale. Since these models have to be considered as limiting cases, any intermediary situation would take place in the corresponding grey zone. When k_{T2} exceeds k_{T1} value, half-reaction time is greater for model 3B than for model 3C. An opposite behaviour is obtained for $k_{T2} = k_{T1}$ (absence of cooperativity).

dimer PrP^{Sc}–PrP^{Sc}) values can rightfully be expected? We saw that cooperativity may come from two cumulative, distinct origins. In parametric terms, cooperativity in the assembly process acts on K_M whereas cooperativity resulting from the modification of subunit interactions upon conformational changes acts on the catalytic constant k_{cat}. When both factors are favourable (increase of k_{cat} and decrease of K_M), their effects are multiplying in k_T. For instance, when each of these parameters is improved by a factor 10, the second-order rate constant k_T corresponding to dimeric catalysis is increased by a factor 100 compared to catalysis by the monomer PrP^{Sc}. Such a rate enhancement would be much more difficult to expect in classical allosteric systems.

Fig. 3 shows the benefit, in terms of catalytic efficiency, resulting from a cooperative dimeric catalysis compared to monomeric catalysis, as it occurs in the Prusiner mechanism. Half-time of conversion is decreased by a factor from 10 to 60 (depending on the model) when dimeric catalysis is assumed to be 100-fold more efficient than monomeric catalysis. Yet larger amplification factors would be expected for any oligomeric complexes having higher order interactions than trimer.

2.4. Cooperative amplification and linear autocatalysis

Fig. 4 compares the relative importance, in terms of catalytic efficiency, of cooperative amplification and linear autocatalysis (which is present both in cooperative and non-cooperative models since it results from the fact that the product of the reaction is also a catalyst). All the numerical calculations were performed assuming the same initial ratio between the concentrations of PrP^C and PrP^{Sc} isoforms. When linear autocatalysis decreased by a factor 100 the half-time of the reaction (compared to a simple exponential process, which corresponds to the case where the product is not also a catalyst of the reaction), an additional effect of a similar amplitude may be expected for a cooperative amplificaton factor (k_{T1}/k_{T2} ratio) of 100. On the contrary, when this ratio does not exceed 5, the benefit resulting from the cooperative effect is marginal compared to the linear autocatalytic effect.

When dimeric catalysis is not cooperative ($k_{T1} = k_{T2}$), the over-all conversion process is slower in the models of multimeric catalysis than in the Prusiner model. This results from the fact that, at the very beginning of the reaction, increase in concentration of the active catalyst is immediate in the momomeric mechanism, whereas it is partially delayed in the other models, leading to a longer lag-time period. This paradoxical effect might have some importance for the understanding of the species barrier phenomenon [23–26].

2.5. Homo- or hetero-catalytic oligomer?

From the results above, we can easily anticipate the behaviour of a system in which the heterodimer PrP^C–PrP^{Sc} would be the effective catalyst, as suggested by Come et al. [15]. Although the kinetic scheme would slightly differ from those we have analyzed, large cooperative amplification factors would be much more difficult to obtain for an heterodimer. Since, in such a case, monomeric PrP^{Sc} has to be assumed to have no catalytic activity (otherwise we find again the schemes we have considered above), only the cooperative effect occurring at the assembly steps may be expected. Hence, the binding of the first PrP^C subunit on the PrP^{Sc} isoform would have to considerably enhance the binding of the second PrP^C subunit. Although we cannot rule out such a possibility, it seems more improbable, from a thermodynamical viewpoint, than that discussed in the case of the homodimer PrP^{Sc}–PrP^{Sc}.

3. Discussion

Thermodynamical considerations show that possible cooperative interactions occurring in a multimeric, catalytic assembly of the pathogenic isoform PrP^{Sc} of the prion protein, could significantly reinforced the autocatalytic character of the process of conformational change which is supposed to be the fundamental event in prion diseases. Such a strengthening is required to explain, according to the Prusiner idea, the kinetics of propagation of prion diseases. However, it should be noted that an alternative mechanism (the nucleation model) of pathogenic prion formation has been proposed [27–29]. Although this model seems to have similarities to our

cooperative model in the sense that it requires assembly of PrP^{Sc} subunits in a polymeric structure, its thermodynamic foundations are quite different [11] because nucleus formation corresponds to a series of thermodynamically unfavorable bimolecular steps.

By adding cooperative interactions to the mechanism of Prusiner, not only the model can work over a more meaningful range of parameter values but also its dynamic behaviour is quite different. As previously shown [12–14], such a positive feedback loop endows the system with bistability properties, i.e. prion propagation would correspond to a switch between a normal and a pathologic, alternative stable steady-state. For instance, if we retain the model of Fig. 3C and leave the simplifying assumption of a simple second-order process, the rate law of conversion between normal and pathologic isoforms of the prion protein can be written as:

$$v = k_s[PrP^C] + \frac{k_{cat}[PrP^C]}{K_M + [PrP^C]}[PrP^{Sc}]^2$$

in which k_s is the first-order rate constant corresponding to the spontaneous conversion process. As shown by Kacser and Small [14], such a formulation is kinetically equivalent to the phenomenological Hill-like equation we previously used [12,13] to describe the dynamic properties of the system.

An important difference between the static view and the dynamic one based on the existence of bistability is as follows: the difficulty in the static mechanism of Prusiner is to find realistic values of kinetic parameters such that PrP^{Sc} would be formed sufficiently slowly by spontaneous conversion to prevent starting of the exponential 'avalanche' of the autocatalytic mechanism of infection. On the contrary, it is not necessary for the PrP^{Sc} isoform concentration to be zero in a non-pathogenic cell, in a bistable system. It is sufficient for this concentration to remain below a bifurcation threshold near the pathogenic steady-state. As a consequence, the rate constant corresponding to the spontaneous conversion process may be several order of magnitude larger in dynamic conditions than that infered in the case of the explosive model [11].

Although much of the experimental evidence supporting the 'protein only' hypothesis were provided by Prusiner and his colleagues, the very concept of a possible 'self-replication' of the prion protein was formulated earlier by the late mathematician J.S. Griffith [9]: "Self-replication need not involve any very intricate mechanism, provided that suitable components are available". Among the components, Griffith emphasized the presence of oligomeric forms of the protein. Thirty years later, our microscopic and mechanistic thermodynamical considerations agree with Griffith's original proposal.

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