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Biophysical Chemistry 72 (1998) 211–222

Biophysical Chemistry

Bistability and the species barrier in prion diseases: stepping across the threshold or not

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Revision received 16 January 1998; accepted 13 February 1998

Abstract

The infectious agent of transmissible spongiform encephalopathies is thought to be a cellular protein, the prion protein, which undergoes, under some circumstances, a dramatic conformational change leading to pathogenesis. The conversion between the normal and pathogenic isoforms corresponds to a autocatalytic mechanism and the metabolism of the prion protein exhibits switches between a normal, stable steady state and a pathogenic one. When the disease can be transmitted between two species, a primary infection from a heterologous donor has to be followed by two passages in the same host species so that the incubation period is stabilized. Sometimes, no pathogenic isoform of the prion protein is detected after the first passage, although corresponding brain extracts remain infectious. The observation that three and only three passages are needed in order to stabilize the strain strongly suggests that, during the course of the primary infection by the heterologous donor, an intermediary conformational species is formed. Within this assumption, a common mechanism involving only conformational changes of the prion protein can give a unifying interpretation of the problem of species barrier, lag characteristics and apparent lack of detection of the pathogenic isoform after the first passage in experiments dealing with interspecies transmission of prion diseases. © 1998 Elsevier Science B.V. All rights reserved

Keywords: Prion diseases; Autocatalysis; Bistability; Species barrier; Strain variations

1. Introduction

A wealth of biochemical and genetic evidence [1–4] supports the contention that a cellular protein (the prion protein) is key for infectivity and propagation of transmissible spongiform encephalopathies (or prion diseases). The dramatic conformational change in the structure of the prion protein is believed to be the fundamental event leading to these fatal, neurodegenerative disorders. The conformational transition involves the loss of substantial alpha-helical content and the acquisition of beta-sheet structure [5,6]. According to the leading theory (known as the 'protein only' hypothesis [1]), the pathogenic, conformational isomer PrP^{Sc} of the normal prion protein PrP^{C} would multiply by converting the normal, cellular isoform into a copy of itself. Inherited prion diseases in humans (which are autosomal dominant disorders) are linked to one of a number of mutations in the *PrP* gene. Mutations would increase the frequency of the

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PII S0301-4622(98)00135-5

spontaneous conversion of PrP^{C} into PrP^{Sc}, allowing the expression of the disease to occur within the lifetime of the individual [1]. Sporadic diseases would also arise from the spontaneous conversion of prion protein from the normal to the pathogenic isoform, due either to a *PrP* gene somatic mutation or to rare instances involving modification of wild-type PrP^{C} protein. In both cases the initial conversion is thought to be followed by autocatalytic propagation [2].

However, Eigen argued [7] that quite unrealistic values of rate parameters are needed in order to allow such an explosive mechanism to occur: the catalytic enhancement (ratio of spontaneous versus catalyzed rates) has to be larger than 10^{15}. We are not aware of any non-cooperative enzymatic turnover that realizes such a rate enhancement. I recently showed [8] that these limits could be overcome by assuming that catalysis proceeds through a multimeric assembly of the pathogenic isoform of the prion protein. Such an assumption agrees with various experimental data, particularly concerning the minimum size of infectious particles [9,10]. An oligomeric 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 endows the metabolic system with dynamic bistability [11–13]. Hence, infection would correspond to a switch between two alternative stable steady states. A similar theoretical analysis was performed simultaneously by Kacser and Small [14].

Rodent adapted models and transgenic animals constitute powerful tools for the study of prion diseases. About 30 different scrapie strains have been isolated so far in mice, depending how rapidly infection takes place and also how the patterns of symptoms and brain lesions are observed [15]. The problem of prion strains is closely related to the so-called species barrier, which hinders (at least under some circumstances) prion transmission from one host species to another [16–20]. In the 'protein only' hypothesis, strain differences would correspond to different conformational or aggregation states of the pathogenic PrP^{Sc} isoform. Transgenic studies argue persuasively that the 'species barrier' is due to differences in *PrP* gene sequences among mammals [21]. Experiments dealing with hamster-adapted mink strains [22] and human prions in a mouse model [4] strongly reinforced the idea that an abnormal prion protein can confer its abnormal conformation onto the host prion protein and that prion diversity is encrypted in the conformation of PrP^{Sc}. However, Lasmézas et al. [23] reported recently that in some cases, no pathogenic isoform of the prion protein is detected upon primary infection of mice with bovine prions, although corresponding mice brain extracts remain infectious for subsequent passages. One could interpret this result to suggest that the prion protein might not be the one and only primary agent responsible of prion diseases. However, this need not be correct.

In this paper, I review the main aspects of the possible occurrence of a bistable behavior in the metabolism of the prion protein. Thereafter, I show that a common mechanism involving only conformational changes of the prion protein can give a unifying interpretation of the problem of species barrier, lag characteristics and apparent lack of detection of the pathogenic isoform after the first passage in experiments dealing with interspecies transmission of prion diseases.

2. The postulated mechanism of prion diseases corresponds to a bistable system

2.1. Biological background

In a cell, the normal prion protein PrP^{C} turns over whereas PrP^{Sc} isoform does not. It is one of the important differences that was evidenced between the two isoforms of the protein. Normal PrP^{C} undergoes endocytosis [24,25] and possibly recycling [26]. The synthesis and degradation of PrP^{C} are rapid with half-time estimated to be 0.1 and 5 h, respectively [24,25]. In contrast, no degradation pathway is known for the PrP^{Sc} isoform and it is probably why this molecular species accumulates in cells once it is formed: PrP^{Sc}, unlike PrP^{C}, gives rise to cerebral amyloid formation, a highly ordered protein aggregate characterized by its insolubility and fibrillar structure [14].

Both experimental [2,27] and theoretical lines of evidence [7,8,11–14,28] have shown that PrP^{Sc} could act as a cooperative, multimeric assembly in the catalyzed conversion between normal and pathologic forms of the prion protein.

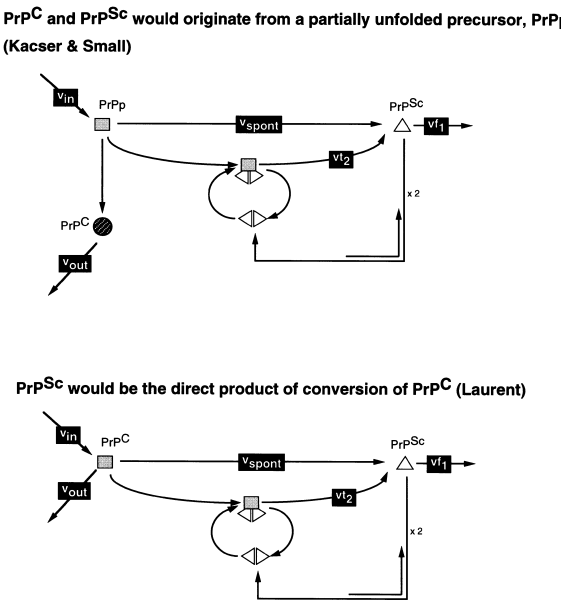

Fig. 1. Bistable schemes in the metabolism of the prion protein. In the Kacser and Small's model, a partially unfolded precursor PrPp of the prion protein is supposed to have two alternative metabolic pathways: either it folds normally, leading to the cellular isoform of the prion protein, PrP^C, or it takes a pathogenic pathway to give the misfolded PrP^{Sc} isoform. In the Laurent model, the normal isoform PrP^C of the prion protein can be changed directly to give PrP^{Sc} and the alternative pathway corresponds simply to the turnover of the normal protein. In both schemes, the pathogenic pathway has two components: a spontaneous (and very slow) reaction (v_{spont}) and a catalyzed one (v_{t2}) which corresponds to an autocatalytic step (catalyst is supposed to be the dimeric form of the pathogenic isoform PrP^{Sc}).

2.2. A bistable scheme

Fig. 1 shows two metabolic schemes that have been proposed independently to account for the propagation of prion diseases. Although they differ slightly in structure, both schemes correspond to open systems that take into account the turnover rate of the normal isoform of the prion protein, PrP^C. In the simplest interpretation, v_{in} and v_{out} steps correspond respectively to the synthesis and degradation of the cellular prion protein. Alternatively, they could represent the rates of entrance and exit into a specialized compartment (endocytosis vesicles for instance) in which the conversion into the pathogenic isoform would occur. In both schemes, one particular step (the conversion of PrPp or PrP^C into the pathogenic isoform PrP^{Sc}) corresponds to an autocatalytic reaction with a non-linear amplification factor. The spontaneous conversion v_{spont} is assumed to be a very slow first-order reaction. By adding cooperative interactions that reinforce the autocatalytic character of the basic mechanism of conversion, both systems have a bistable behavior. For instance, in Laurent's scheme, the evolution of the concentrations of the species over time is described by the following differential equations (which apply, with some obvious modifications, for Kacser and Small's scheme as well):

$$\frac{d[PrP^C]}{dt} = v_{in} - v_{out} - v_{spont} - v_{t2} \quad (1)$$

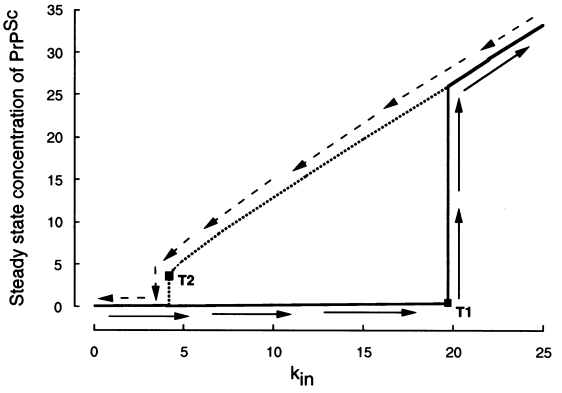

Fig. 2. Hysteretic transitions between stable steady states upon variation of the turnover rate of the PrP^C protein. Let us suppose the system is initially in the non-pathogenic steady state (low PrP^{Sc} concentration) and that a continuous increase in the rate k_{in} of PrP^C formation occurs (straight arrows). Until the stationary state lies on the lower branch of the corresponding PrP^{Sc}-nullcline, PrP^{Sc} concentration is slightly readjusted, in accordance with the solution trajectory. But when the variable parameter becomes greater than a threshold value T_1, the system moves to the upper branch of the PrP^{Sc}-nullcline and a strong, sudden increase appears for the steady state concentration of PrP^{Sc}. If k_{in} parameter is now decreasing (dashed arrows) while the system is on the higher branch of stability, the system moves down on this branch until the slope of the nullcline begins negative (T_2 threshold). Then a switch occurs towards the lower branch of the PrP^{Sc}-nullcline. Hence, the values of k_{in} for which switches between the alternate steady states occur are different depending on whether this parameter increases or decreases (hysteretic transitions). The same dynamic behavior is observed when the rate constant k_{out} of PrP^C degradation is chosen as the variable parameter. In all 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. Parameter values are: $k_s = 0.06$, $k_{cat2} = 0.1$, $K_{M2} = 1$, $k_{out} = 7.5$, $k_{f1} = 0.75$, $k_{ass0} = 0.01$, $k_{diss0} = 100$.

$$\frac{d[PrP^{Sc}]}{dt} = v_{spont} - v_{t2} - v_{f1} \quad (2)$$

Assuming a rapid equilibrium for dimerization of the PrP^{Sc} isoform, the rate law v_{t2} can be written as [8]:

$$v_{t2} = \frac{k_{cat}[PrP^{C}]}{K_M + [PrP^{C}]}[PrP^{Sc}]^2 \quad (3)$$

Other steps are considered as corresponding to first-order kinetics (except v_{in} which is a zero-order step).

Analysis of the null isoclines of this two variable system (see Refs. [11,12] and insert in Fig. 6) shows the existence of two distinct branches of stability of the steady states. When two alternate, stable steady states coexist, they are associated with low (normal steady state) and high (pathogenic steady state) stationary concentrations of the pathogenic isoform PrP^{Sc} of the prion protein. As shown in Fig. 2, the rate of PrP^{C} synthesis (or, more generally, the turnover rate of the normal isoform PrP^{C} of the prion protein) may govern the hysteretic switches between normal and pathogenic steady states.

As a consequence of this dynamic behavior, characteristics of prion infection and propagation differ from those of virus replication upon several crucial points: (1) healthy organisms are able to eliminate spontaneously infrathreshold amounts of foreign PrP^{Sc} protein; (2) under controlled conditions, additions of suprathreshold quantities of PrP^{Sc} or also of the normal PrP^{C} protein, provoke a transition towards the pathogenic steady state; (3) in a healthy organism, the concentration of the PrP^{Sc} isoform is below a threshold value but is not necessarily null.

2.3. Comparison with available experimental data

The presence of a small amount of the PrP^{Sc} protein in lymphocytes was reported as prevalent in the human population [29]. Our results show that this possible presence does not constitute necessarily any indication for a non-symptomatic but infectious pathogenic state. Moreover, infectious prion particles should not be seen as necessarily composed only of the abnormal isoform of the protein, as usually stated. Theoretical analysis shows that particles containing overproduced normal PrP protein might also be pathogenic. Convergent data [19,30,31] demonstrate that the disease progresses much more slowly in heterozygous mice (*Prn-p*$^{0/+}$ mice having one normal allele for the *PrP* gene and one allele in which the *PrP* gene is disrupted) than in wild-type controls (*Prn-p*$^{+/+}$). The difference in incubation times between transgenic and wild type mice is about a factor of 2 for the agent of the mouse-adapted Creutzfeldt–Jakob disease (CJD) agent [31] and exceeds a factor of 10 in the case of the scrapie agent [19,30]. If we compare incubation times for *Prn-p*$^{0/0}$ (homozygous for the disrupted *PrP* gene) *Prn-p*$^{0/+}$ and *Prn-p*$^{+/+}$ mice, normal PrP^{C} seems to afford some kind of partial protection against scrapie or CJD disease. However, in the framework of the 'protein only' hypothesis, these data indicate that the susceptibility to scrapie or CJD is a function of the level of normal PrP^{C} in the host, in accordance with the dynamic analysis.

Until now, the existence of scrapie strains and the problem of the species barrier have been considered as the weak part of the 'protein only' hypothesis which assumes that the prion protein would be by itself the agent responsible of the propagation of prion diseases. Can we imagine some realistic mechanism able to stabilize distinct phenotypes of the prion protein and to interpret the kinetic characteristics of the species barrier?

3. Conformational changes of the prion protein and transmission of the disease between species

3.1. Lag characteristics, species barrier and the 'thre e passages' rule

When possible, transmission of prions from one species to another occurs only after prolonged incubation times [6,7]. For instance, in the experimental infection of two closely related mustelids with the mink agent [20], a longer incubation period was observed in ferrets (28–38 months) than minks (4 months). As a rule, inoculation of mice brains with different prion species [32,33] shows that the incubation period diminishes between the primary infection from a heterologous donor and the period in subsequent passages in the same mouse strain. When transmission can occur, three passages are generally needed before the strain is stabilized (i.e. no further decrease in the incubation period is observed for subsequent passages). Sometimes, the disease even does

not break out and no pathogenic isoform of the prion protein is detected after the first passage, although corresponding brain extracts remain infectious [23].

3.2. A model for the conformational adaptation of the protein

Fig. 3 summarizes a very simple mechanism based on conformational changes of the prion protein and that can account with the lag characteristics observed during propagation of the diseases. The general observation that three and only three passages are needed in order to stabilize the strain strongly suggests that, during the course of the primary infection by an heterologous donor, an intermediary conformational species, PrP*, is formed. This species would be different from both the PrP^{Sc} isoform in the inoculum (*inoc*PrP^{Sc}) and also the final, stabilized PrP^{Sc} isoform (*host*PrP^{Sc}) found in the host, after the third passage. At least two functional differences may be expected between PrP* and *inoc*PrP^{Sc}: either PrP* has a low catalytic constant (compared to that of *inoc*PrP^{Sc}) for the conversion of host PrP^{C} into *host*PrP^{Sc} or the association of PrP* into its oligomeric, catalytically active form is thermodynamically unfavorable. The second part of the alternative has to be privileged, otherwise the strain would be stabilized at the second passage. Accordingly, the preferential catalyst for the conversion of host PrP^{C} during the first passage would be the oligomeric form of *inoc*PrP^{Sc}, so that essentially PrP* form would be produced at that time. Because of the absence of close homotypic relationship between exogenous *inoc*PrP^{Sc} and host PrP^{C} protein, catalytic efficiency during the first passage is low and the conversion between PrP^{C} and PrP* occurs only after prolonged incubation times.

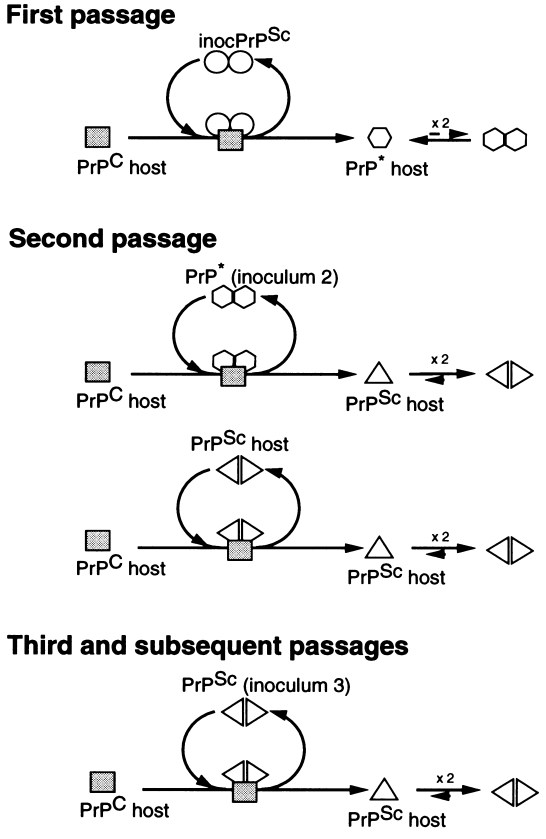

Fig. 3. A plausible Prusiner-like model interpreting adaptation of prion conformation between species. Conversion of the normal prion protein PrP^{C} is assumed to be catalyzed by an oligomeric form (a dimer in this scheme) of the modified protein, PrP* or PrP^{Sc}. Although the molecular products of the different catalyzed steps are represented by different symbols, no particular assumption has to be made concerning the extent of conformational differences between the corresponding species. The pertinent parameters are only the relative values of the catalytic constants and the low value of the equilibrium constant for the association of the intermediary species, PrP*.

When mice are inoculated with extracts prepared from brain tissue obtained after the first passage, the main species found in the inoculum is the monomeric, catalytically inactive PrP* isoform and, to a lesser extent, the corresponding multimeric, catalytically active form. The catalytic PrP* protein produces the final, stabilized PrP^{Sc} isoform (*host*PrP^{Sc}). Hence, we should observe, in the second passage, a lag period corresponding to the time needed to produce significant amounts of *host*PrP^{Sc}. However, this species easily forms catalytically active, oligomeric structures so that, during the second passage, conversion of PrP^{C} would essentially act, after the lag period, through catalysis by *host*PrP^{Sc}. After the second passage, catalytically active, oligomeric form of *host*PrP^{Sc} is present and it will constitute the main species found in extracts prepared for the next inoculation. Hence, during the third and subsequent passages, the infection will start with a considerable complement of *host*PrP^{Sc} and there will be no further change in the incubation period.

Possible development of the disease after the first passage only depends on the intrinsic pathogenicity of

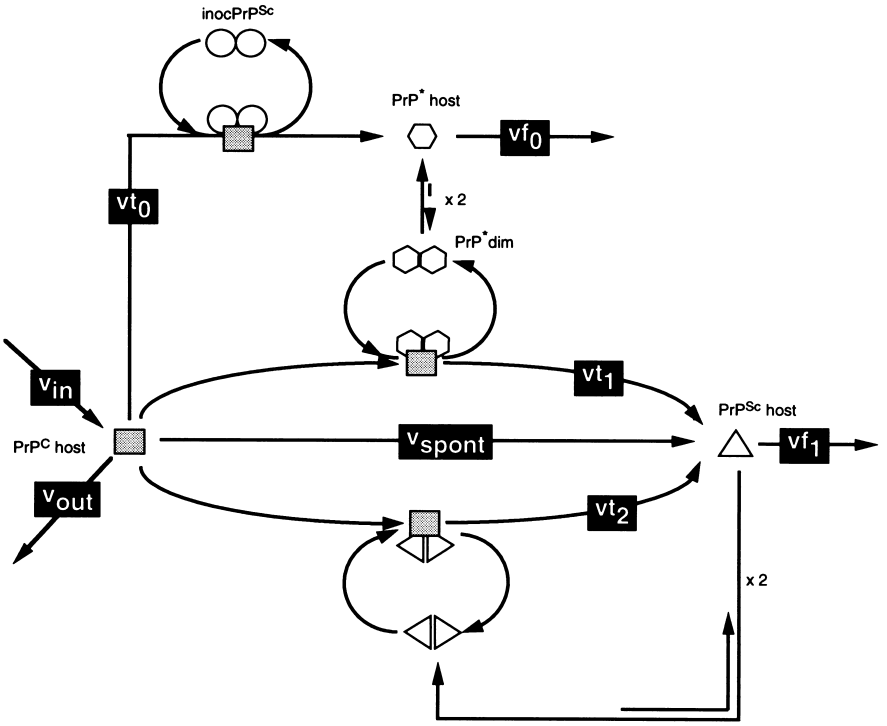

Fig. 4. Kinetic scheme associated to the model of Fig. 3. Individual steps correspond to the following rate equations:

$v_{in} = k_{in} \quad v_{out} = k_{out}[PrP^C] \quad v_{f0} = k_{f0}[PrP*] \quad v_{f1} = k_{f1}[PrP^{Sc}] \quad v_{spont} = k_s[PrP^C]$

$$v_{t0} = \frac{k_{cat0}[inocPrP^{Sc}][PrP^C]}{K_{M0} + [PrP^C]}$$

$$v_{t1} = \frac{k_{cat1}[PrP*dim]PrP^C]}{K_{M1} + [PrP^C]}$$

with $[PrP*dim] = k_{ass}[PrP*]^2 / k_{diss}$

$$v_{t2} = \frac{k_{cat}[PrP^C]}{K_M + [PrP^C][PrP^{Sc}]^2}$$

Assuming a rapid equilibrium for the dimerization step of the Prp^{Sc} species, the evolution of the concentrations of the species over time is described by the following differential equations:

$$\frac{d[PrP^C]}{dt} = v_{in} - v_{out} - v_{spont} - v_{t2}$$

$$\frac{d[PrP^{Sc}]}{dt} = v_{spont} + v_{t2} - v_{f1}$$

$$\frac{d[PrP*]}{dt} = v_{t0} - v_{f0} - k_{ass0}[PrP*]^2 + k_{diss0}[PrP*dim]$$

the PrP* form. No particular requirement about this point is needed in this model. Moreover, such a mechanism easily interprets that, in some cases, no pathogenic isoform of the prion protein is detected after the first passage, although corresponding brain extracts remain infectious. The possibility of detecting PrP* isoform only depends on the differences between PrP* and *host*PrP^{Sc} for the experimental criterion used, i.e. it depends on the relative resistance of the various isoforms of the protein towards proteinase K. No particular assumption has to be made in this model concerning the sensitivity of the postulated PrP* isoform towards proteolysis.

Do these conclusions remain valid in a dynamic

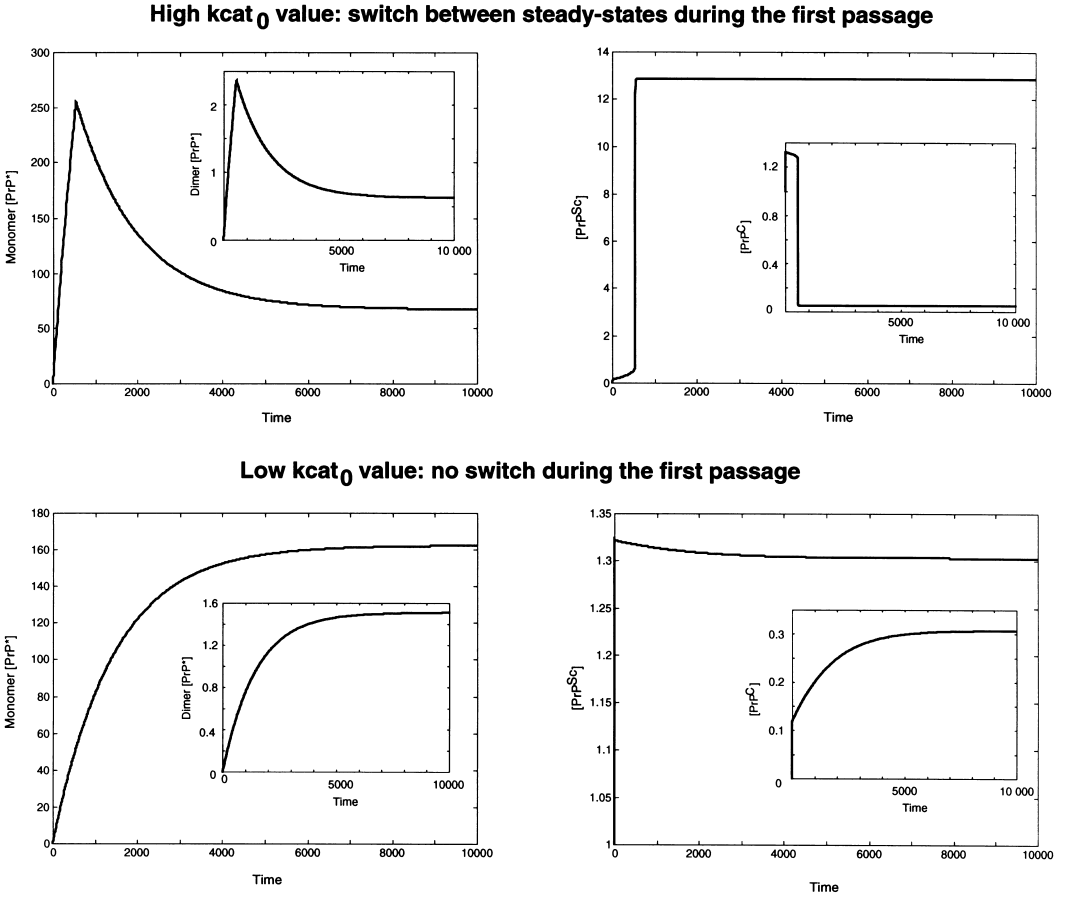

Fig. 5. The transition between alternative steady states may or may not occur after the first passage, depending particularly upon the value of k_{cat0}. (Upper) $k_{cat0} = 0.005$. At about $t = 500$ units after primary inoculation with prion from a heterologous donor (*inoc*PrP^{Sc}), the system switches towards a steady state having the characteristics of the pathogenic steady state (low [PrP^{C}] and high [PrP^{Sc}] values). The transition occurs when the concentration of the PrP* (monomeric or dimeric) intermediary isoform reaches a suprathreshold level. (Lower) $k_{cat0} = 0.001$. Monomeric and dimeric isoforms of PrP* species reach a stationary level slowly, which is lower than the threshold level. Hence, the switch between alternative steady states does not occur and the system remains, after the first passage, in a steady state having low [PrP^{Sc}] value (compare concentration scales in upper and lower panels). In these conditions, PrP^{Sc} isoform is not detected after the first passage and development of the disease in the infected organism will depend only on the intrinsic pathogenicity of the PrP* isoform. Note that the steady state characteristics of such an infected organism in which the switch has not occurred are quite different from those found in an uninfected organism (particularly concerning the stationary level of the PrP* isoform). Data were obtained from numerical integration of the differential equations corresponding to the scheme of Fig. 4 with the following values of parameters: $k_s = 0.06$, $k_{cat2} = 1.25$, $K_{M0} = 1$, $K_{M1} = 1$, $K_{M2} = 1$, $k_{in} = 10$, $k_{out} = 7.5$, $k_{f1} = 0.75$, $k_{cat1} = 0.1$, $k_{f0} = 0.075$, $k_{ass0} = 0.01$, $k_{diss0} = 100$, [*inoc*PrP^{Sc}] = 200. Initial concentrations of the species where 0, except for PrP^{C} ([PrPC]$_0$ = 1).

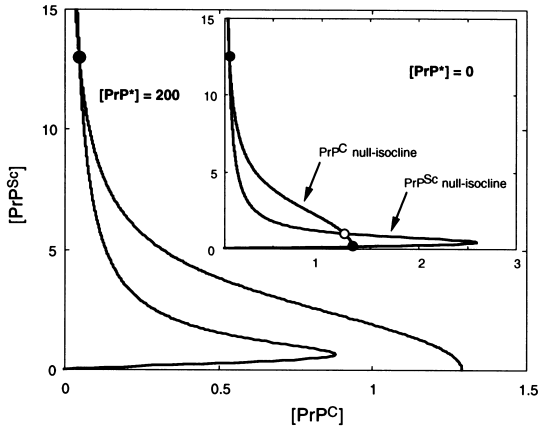

Fig. 6. Null isoclines associated with a simplified version of the scheme in Fig. 4 and corresponding to the dynamics observed during the second passage. To simulate the conditions which prevail during the second passage (see text), we choose [*inoc*Pr P^{Sc}] = 0 and $[PrP^*]_0 = 200$ ($k_{ass} = 0.01$, $k_{diss} = 100$ for the rate constants corresponding to the equilibrium between the monomeric and dimeric PrP^{Sc} isoforms and other parameter values as in Fig. 5. Neglecting the slow leak term v_{f0}, [PrP*] is now a constant and this simplified version of the scheme in Fig. 4 corresponds to a two variable system. In the absence of infection (insert, $[PrP^*]_0 = 0$), the sigmoid PrP^{Sc} null isocline has three intersections with the monotonic PrP^C null isocline, in accordance with previous descriptions of a very similar bistable system [11–14] (having slightly different rate equations). Normal mode analysis of the corresponding equations shows that the stable steady states are stable nodes and the unstable steady state is a saddle point. The presence of the intermediary isoform PrP* modifies profoundly the shape of the sigmoid PrP^{Sc} null isocline (whereas the shape and the position of the PrP^C null isocline are not significantly changed), so that the two nullclines have only one intersection. The system is now globally stable and the steady state corresponds to the pathogenic steady state (high level of PrP^{Sc} isoform). Hence, upon inoculation of brain extract in the second passage (the inoculum is mainly constituted of highly concentrated PrP* isoform; see Fig. 5), the system tends towards a pathogenic steady state, even if no transition has occurred during the first passage.

context which takes into account the turnover rate of the normal prion protein?

3.3. Dynamic component in the species barrier problem

Analysis of the kinetic scheme (Fig. 4) corresponding to the descriptive model of Fig. 3 shows that the switch between the normal and the pathogenic steady states may or may not occur upon the primary infec-

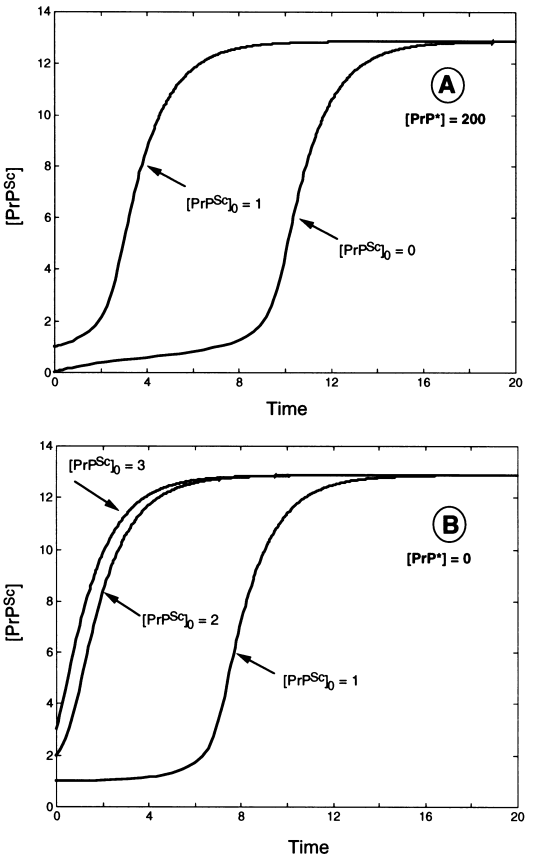

Fig. 7. Differences in lag times observed between the second and third passages in the course of the transmission of prion diseases between two species. For the second and third passages, the structure of the kinetic scheme is as described in Fig. 6 (simulations were performed with the same values of the parameters as in Fig. 5). However, initial values of the variables are not identical in both cases. For the second passage, the system starts with a high level of PrP* and a very low level of PrP^{Sc} (if the transition between steady states has not occurred during the first passage). This corresponds to the curve $[PrP^{Sc}]_0 = 0$ in (A). For the third passage, the system starts with a rather low level of PrP* (because of dilution) and a rather high (but diluted after inoculation) concentration of PrP^{Sc}. In fact, calculations show that the half-time effect is not significantly different for curves obtained for $[PrP^{Sc}]_0 = 1$ and $[PrP^*] = 200$ (A) on the one hand and $[PrP^{Sc}]_0 = 2$ (or larger) and $[PrP^*] = 0$ (B) on the other hand. On the contrary, the half-time effect is lower than that observed during the second passage (curve $[PrP^{Sc}]_0 = 0$ in (A). Hence, the occurrence of some kind of variations between organisms for the values of the steady state concentration of the various protein isoforms obtained after the second passage, will not influence significantly the lag characteristics corresponding to the third passage. Obviously, subsequent passages would give results identical to those observed during the third one, i.e. the disease will be stabilized in the host species.

tion, depending (among other kinetic parameters) on the value of the catalytic constant of the PrP^{Sc} isoform in the inoculum (Fig. 5). However, even when the transition does not occur, the characteristics of the normal steady state are considerably modified with respect to those which prevail in the absence of infection. The levels of PrP^{C} and *host*PrP^{Sc} isoforms remain respectively high and low, but the amount of PrP* isoform (monomer and dimer) may be important (unless *inoc*PrP^{Sc} does not catalyze significantly the formation of PrP*, leading to a situation in which the barrier between the two species cannot be overcome).

Extracts prepared from brain tissue obtained after the first passage merely contain the monomeric and multimeric PrP* isoforms. These are diluted upon inoculation introduced by the second passage. Then the question arises as to why the switch between normal and pathogenic steady states does occur at this stage and not at the end of the first passage, although these species are much more concentrated. In fact, the structure of the kinetic scheme is slightly different from the one which prevails during the first passage. Now in effect there is no way to convert PrP^{C} into the intermediary isoform PrP* since the corresponding catalyst (*inoc*PrP^{Sc}) is virtually absent in this second passage, because of the dilution effect. As a result, both the initial conditions and the characteristics of the steady state of the system could be quite different between the first and the second passages. Fig. 6 shows that the presence of the PrP* isoform may suffice to modify the shape of the null isoclines (compared to the situation in which there is no infection), so that only the pathogenic state exists after the second inoculation. In these conditions, the lag characteristics would only correspond to the time needed by the system to reach its globally stable steady state, starting from initial conditions in which the concentration of the PrP^{Sc} isoform is low.

The structure of the kinetic scheme corresponding to the third passage is identical to that described for the second passage, but the initial conditions are quite different. Now, we start with a high, suprathreshold concentration of PrP^{Sc} isoform and a low concentration (due to the dilution) of PrP* isoform. Since the catalytic constant corresponding to the PrP^{Sc}-catalyzed process is supposed to be better than that associated with the PrP*-catalyzed process, the pathogenic (Fig. 7) steady state will be reached more rapidly than it is observed during the second passage. Subsequent passages will start with initial conditions that are identical to those described for the third passage, so that no further change in the incubation time is observed.

4. Discussion

The developing body of knowledge concerning prion diseases indicates that something unusual, and not easily explainable by familiar ‘orthodox’ mechanisms, is operating in these situations. Originally thought to be ‘unusual or slow viruses’, the infectious particles seem to be devoid of any detectable nucleic acid. However, since they are infectious, these particles must be somehow capable of reproduction. There is now a wealth of biochemical and genetic evidence supporting the contention that a cellular protein (the prion protein) is responsible for infectivity. Today, although some authors still believe that the infectious agent is a specific nucleic acid associated with or packaged in some host-derived protein [34–36], the most accepted theory in the field, known as ‘the protein-only’ hypothesis [1], postulates that the conversion of the constitutive host protein PrP^{C} to a pathogenic, isomeric form PrP^{Sc} is the molecular basis of prion diseases.

The conformation of a protein can be influenced by complex interactions with many other cellular components. Hence, we cannot exclude, at the present time, a possible intervention of another component in the propagation of the disease. Such a question pertains to experimental fields. On the other hand, since experiments have failed so far to find such an additional element, an important question which is relevant for modeling activity may be asked: is it quite conceivable, from a mechanistic point of view, that the ‘protein only’ hypothesis can give a complete explanation for all the observations reported about prion diseases? Previous theoretical studies [7,8,11–14] have shown that the purest form [1] of this elegant and provocative hypothesis has to be improved in order to account for the kinetics of the propagation of the diseases. The mechanism of Prusiner can work over a meaningful range of parameter values if one assumes an oligomeric structure for the proteinaceous catalyst and the existence of cooperative interactions

between subunits. These modifications then alter the dynamic behavior of the model. Such a positive feedback loop endows the system with bistability properties so that prion propagation corresponds to a switch between a normal and a pathogenic, alternative stable steady state.

Until now, the existence of the species barrier and the observation that brain extracts can be infectious despite the apparent lack of the pathogenic isoform of the prion protein, have been considered as the weak part of the 'protein only' hypothesis. In this paper, I have shown that quite reasonable hypotheses about the adaptability of the prion protein allow us to give a unifying interpretation to conformational diversity and lag characteristics observed during transmission of prion diseases between species. This model involves only conformational changes of the protein. Although a complete parametric study of this model remains to be made, our preliminary results indicate that it can describe, in a dynamic context, the 'three passages' rule and also the experimental fact that, in some cases, no pathogenic isoform of the prion protein is detected upon primary infection of mice with bovine prions, although corresponding mice brain extracts remain infectious for subsequent passages. One should note that the mechanism postulated here can be tested by inoculating host PrP^C in mice brain that do not become ill after the first passage. This model anticipates that the mice would develop the disease after this second injection.

Beyond the problem of the species barrier, the existence of multiple strains of the transmissible agent has been considered as an indication that these diseases might be caused by a replicating agent with an independent genome. Many of the changes found in mouse strains can be interpreted by the same type of argument as that given above for the adaptation of various prion diseases to different species. However, it would seem unreasonable to imagine that the mouse prion protein can exist under about 30 different conformations (this is the number of mouse-adapted scrapie strains identified so far), as it is inferred by the simplest interpretation of Prusiner's mechanism. On the contrary, combining several conformations and/or oligomerization states of the pathogenic forms of the prion protein, can conceivably lead to the emergence of multiple 'strains' of the proteinic agent, i.e. to macromolecular structures having their own catalytic characteristics. As previously stated [37], most of the variance in incubation times can be explained in terms of interactions between donor (the animal from which infectious material is extracted for injection in the host) and host prion protein. The possibility that the host protein may influence changes induced by the inoculated agent is consistent with the concept that 'strain of agent' is another expression of the involvement of prion protein in the mechanism of pathogenesis of these diseases. Hence, a common mechanism involving protein-only conformational changes can give a unifying interpretation of the problem of species barrier, strain variations and lag characteristics observed with transgenic animals.

Does the prion protein behavior constitute a very unusual, pathogenic mechanism specifically bound to this particular protein or does it illustrate a mechanism which might also be involved in other normal proteins to achieve and pass on their functional conformation? Such a process has been invoked in yeast (URE3 and psi determinants) to explain observations about extrachromosomic inheritance controlling phenotypic characters by a non-mendelian way [38,39]. Similarly, a very puzzling observation has been reported about the multifunctional p53 protein. When *p53* mutant and wild type are cotranslated, the mutant protein can drive the wild type into a mutant conformation [40]. The cotranslational effect of mutant *p53* upon wild type conformation was attributed to interactions between nascent polypeptides and oligomerization of the full-length proteins. Oligomers of p53 proteins can be induced to conformational change in a cooperative manner. Nevertheless, the most striking phenomenon with similarity to the mechanism postulated for prion diseases has also been known for a long time, that is the self-assembly of bacterial flagella. Most bacterial flagella are made of a single type of protein, flagellin. The flagellum can be reconstituted in vitro if a small-polymerized 'seed' of flagellin is present in the assembly mixture [41]. A single mutation in the flagellin gene alters the tertiary structure of the protein and the subsequent phenotype of the flagellum, resulting in a 'curly' phenotype. In vitro, reconstituted curly flagellin yields curly flagella. However, when wild type flagellin is reconstituted in the presence of seeds of curly flagella (or the inverse), the reassembled flagella possess the curly phenotype [41,42]. Hence, curly seeds are apparently

capable of inducing wild-type flagellin monomers to the curly structural conformation. Thus, the structural shift to a curly configuration is induced not only at the boundary between the seed of curly subunits and the wild type subunits added to it but, beyond that, from wild type molecule to wild type molecule along the entire extent of the flagellum [43]. Hence, the ability of a protein to impose its own conformation to a distinct one has not to be seen as an unexpected mechanism. It does not obey the central dogma of gene expression but it illustrates that some phenomena may operate beyond the classical paradigm of the transcription-translation sequence. Probably that the prion problem emphasized, at the molecular level, the equal importance of genetic and epigenetic components to understand the concept of structural inheritance and the emergence of the functional structures of life.

Acknowledgements

I am indebted to Dr. Sina Adl for careful reading of the manuscript. This work was supported by grants from the Centre National de la Recherche Scientifique (Appel d'offres: 'Biologie cellulaire, du normal au pathologique') and the Université Paris-Sud.

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