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Prionics or The kinetic basis of prion diseases

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Abstract

A comparative kinetic analysis of mechanisms of prion diseases based on the "protein only" hypothesis is presented. The Prusiner mechanism of autocatalytic conversion of a host protein into a genetically identical, but conformationally different, prion state requires cooperativity in order to work, given realistic values of rate parameters. It then becomes phenomenologically indistinguishable from the Lansbury mechanism of plaque formation which is also a form of (passive) autocatalysis. Though the two kinds of mechanisms still may differ on the question which of the two monomeric protein conformations is the favoured equilibrium state they both require an aggregated state as the form that is eventually favored at equilibrium. While these considerations allow for a critical comparison of the mechanisms they do not yet tell us what the actual mechanism of *infection* is. Experiments rather indicate that the infectious unit *in vivo* may still differ from an *in vitro* form of aggregated prion proteins. Hence aggregation of the prionic form is most probably a necessary, but possibly not sufficient, prerequisite of infection. Be that as it may, the premise of a linkage between prion aggregation and infection offers a very sensitive method for diagnosing the disease at a very early stage, using fluorescence cross-correlation analysis. The possible analogies to Alzheimer's disease make such a prospect a "hot topic".

Keywords: Prion diseases; Autocatalysis; Cooperativity; Plaque formation; Nucleation; Chemical instabilities

1. What is a prion?

The term has been coined in order to distinguish a particular class of pathogens from other infectious agents such as bacteria or viruses [1]. This type of pathogen is held responsible for transmissable spongiform encephalopathies (TSE). The mad cow disease, bovine spongiform encephalopathy (BSE), that has captured the headlines of European newspapers, is presently the most popular (or rather unpopular) example. Others are scrapie in sheep and various forms in man, such as the Creutzfeldt–Jacob and Gerstmann–Sträussler–Scheinker diseases as well as the fatal familial insomnia and kuru. Originally it was a mere speculation [2,3] that the essential (or possibly only) pathogenic compound causing these diseases was a protein molecule, i.e. a prion; however, there is now abundant evidence favoring the "protein only" hypothesis. The infectious protein possesses quite uncommon properties. Physically

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and chemically it is unusually stable; it resists high temperatures (well above the boiling point of water) as well as other denaturing procedures such as formaldehyde treatment or UV irradiation. Genetically it closely resembles — or even is identical to — a naturally occuring form of protein inherent to vertebrate organisms. The mature product of the natural gene, called PrP^c, is glycosylated and fixed to the plasma membrane of neurons and certain other cell types. Experiments show that the presence of this natural protein is necessary for an infection to become effective. The infectious agent is assumed to be a product of the same gene as PrP^c and to differ from the natural form only in its conformation. Accordingly it has been named PrP^{Sc} (Sc for scrapie). Operationally PrP^{Sc} is distinguished from PrP^c by its (partial) resistance to proteinase K digestion (after treatment with detergents). In fact, PrP^{Sc} is the structurally more stable form that is rich in β-sheet structures and aggregates irreversibly at sufficiently high concentrations. This aggregation has been associated with β-amyloid plaque formation as typically present in the brains of patients with Alzheimer's disease [4]. Clinically, prion diseases show an unusually long incubation period of (possibly) many years. Sporadic forms correspondingly have a very low incidence. However, if the disease becomes clinically evident, e.g. by symptoms such as motor perturbations or loss of memory, it progresses rapidly to dementia and inevitably to death. This is a short "wanted" description for the prion, a rather condensed summary which I have distilled from a more detailed review by Weissmann and coworkers that describes excellently the present knowledge of pathogenesis of prions, as well as the state-of-the-art of experimentation with the disease. The review was made available to me prior to publication [5]. As mentioned before, the "protein only" hypothesis now is convincingly based on experimental evidence [6–11]. Originally, it seemed surprising that such a species, devoid of nucleic acid genes, apparently is able to reproduce and multiply in a host like a virus particle.

2. Replication without RNA or DNA?

All infectious agents that multiply and spread in nature are microorganisms such as bacteria and fungi or viruses. The basis of their multiplication is the inherent replicative power of RNA or DNA. For many biologists replication without RNA or DNA seems unimaginable. Therefore, the hypothesis of "protein only" has worried many researchers up to the present day [12]. The sporadic incidence of human prion diseases is very low ($1:10^6$ per year); however, the disease can usually be transmitted experimentally to laboratory animals suggesting that there is an agent that can replicate. There is indeed such an agent, but all experimental evidence shows that it is devoid of RNA or DNA.

How can this be? The answer depends on what we mean by replication. We must distinguish plain autocatalysis, which is a special form of reaction mechanism, from *inherent* autocatalysis which is the basis of replication in biology [13]. Replication, idiomatically, is nothing but reproduction by copying. As such it involves a template and a replica. It is exactly in this sense that the word replication is used in molecular biology. Replication of RNA or DNA means a "symbol-by-symbol" copying that retains the primary structure of a template sequence of nucleotides. It is based on a chemical property, an exclusive affinity between the particular purines and the particular pyrimidines that make up the four classes of nucleotides. This affinity establishes a complementarity between the nucleotides to which the replication enzymes have adapted. The ability to reproduce is inherent to all polynucleotide sequences, be they RNA or DNA. Note the word "inherent" I have introduced with the preceding sentence. We must distinguish "incidental" from "inherent" reproduction. The latter is a sole attribute of nucleic acids. Proteins as a whole are devoid of this property. This does not exclude that we can construct special sequences of polypeptides that are able to reproduce. Very recently such a case has been reported [14], i.e. a peptide sequence that is able to reproduce showing kinetics similar to that of enzyme-free template-instructed oligonucleotide amplification [15].

Moreover, we could — theoretically — construct reaction networks of proteins in which a cyclic closure — i.e. a feedback loop — brings about autocatalysis. These cycles are called Kauffman cycles and are assumed to have been active during nucleic acid-free early chemical evolution. They were analyzed

(prior to their "re-invention" by Kauffmann [16]) and indeed shown to be self-reproductive [13]. They function with very specific structures, but require coincident mutations of all members in the cycle in order to evolve an optimal performance. Autocatalysis as such is necessary but not sufficient for evolution. Selection requires a mechanism of reproduction, but what is required for evolution is "inherent" rather than "incidental" reproductivity.

Viruses have this property; therefore, viral diseases steadily "evolve" (more or less rapidly) [16]. Prion diseases — according to all experimental evidence — progress in an autocatalytic fashion. Upon infection a host factor is converted into the very agent that causes the infection. The word "autocatalysis" is used here without closer specification. As will be seen later, autocatalysis may proceed by an active or a passive mechanism. Nevertheless, in prion infection we are dealing with a specific or "incidental" autocatalytic mechanism. The infectious agent shares a common primary structure with a protein expressed by the host. The "protein only" hypothesis is well supported, to a large extent by the experimental evidence provided by Prusiner [5,17]. Highly purified prion preparations contain less than one molecule of nucleic acid larger than about hundred nucleotides [10,11].

The primary sequences of the proteins involved are determined by the host's genome. The pathological form differs from the naturally produced host form in its secondary, tertiary (and possibly also quaternary) structure. Prusiner first proposed a mechanism for an autocatalytic turnover of the host form into its pathological analogue [18]. The Prusiner model (cf. Section 3) resembles the idea of "induced fit" that provided one of the early descriptions of the impact that conformational changes can have on enzyme catalysis (cf. Section 4). An alternative model representing a more passive catalytic action has been put forward by Lansbury Jr. and coworkers (cf. Section 5). The main purpose of this paper is to present a more rigorous kinetic analysis of these (and alternative) "protein only" models, a more quantitative comparison of such models with experimental results may provide some clues regarding the nature of prion diseases (cf. Section 6).

3. The Prusiner mechanism of autocatalytic conformational change

The following kinetic treatment is based on the mechanism shown in Fig. 1. It contains more steps than mentioned by Prusiner; these extra steps are logically required, even though the values of the rate constants may be negligibly small. For the sake of notational economy it is useful to introduce the following definitions: A is the normal form of host protein, usually called PrP^{c}; B is the pathogenic form of the same protein, the prion PrP^{Sc}; the symbol [ ] denotes concentration.

Rate constants		Rate terms
Non-catalytic conversion A to B:	k_{AB}	$k_{AB}[A]$
Non-catalytic conversion B to A:	k_{BA}	$k_{BA}[B]$
Metabolic decomposition of A:	k_{-A}	$k_{-A}[A]$
Metabolic decomposition of B:	k_{-B}	$k_{-B}[B]$
Metabolic formation of A:		F_A = constant
Autocatalytic formation of B:		$\frac{k_T[A]}{K_M + [A]}[B]$
Turnover number:	k_T	
Michaelis constant:	K_M	

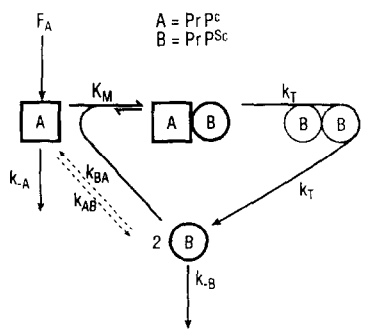

Fig. 1. The Prusiner mechanism of linear autocatalysis. The meaning of states and rate parameters is obvious from the text.

With these definitions we can write down the following rate equations:

$$\frac{d[A]}{dt} = F_A - k_{-A}[A] - k_{AB}[A] - \frac{k_T[A]}{K_M + [A]}[B] \quad (1)$$

$$\frac{d[B]}{dt} = k_{AB}[A] + \frac{k_T[A]}{K_M + [A]}[B] - (k_{BA} + k_{-B})[B] \quad (2)$$

In these phenomenological equations, A and B are assumed to be spatially uniformly distributed. No distinction is made whether they are homogeneously soluble or fixed to neuronal membranes; however, this will be of importance if the rate constants are interpreted by molecular-mechanical models. The usage of a stationary Michaelis–Menten expression in the autocatalytic term is justified since binding processes can be assumed to be fast compared to conversions [19]. The two coupled differential equations contain non-linear terms and therefore can be solved explicitly only for limiting cases as detailed in Appendix A. However, by setting these equations to zero one can obtain two steady states as roots of a quadratic equation (Appendix A). Obvious properties of these solutions follow from a simple inspection of the two implicit forms:

$$[A] = \frac{F_A}{k_{AB} + k_{-A} + \dfrac{k_T[B]}{K_M + A}} \quad (3)$$

$$[B] = \frac{k_{AB}[A]}{k_{BA} + k_{-B} - \dfrac{k_T[A]}{K_M + [A]}} \quad (4)$$

First of all, it should be recognized that we are dealing with a notoriously non-equilibrium situation. A is formed and decomposed metabolically in a non-reversible fashion and so is B. The equilibrium A $\leftrightarrow$ B must favor B, otherwise there would be no driving force for the catalytic turnover which at equilibrium is reversible. Here we are dealing with steady states. In the absence of infection the steady state obviously favors A which is present with its initial concentration $[A]_0$, while we assume B to be (practically) unpopulated. For this to be true the catalytic term in Eq. (4) has to be negligibly small (as compared to $(k_{BA}+k_{-B})$, even when A is present at the highest achievable concentration, $[A]_0$).

The precise explicit solution (Appendix A) then tells us that

$$[A]_0 = \frac{F_A}{k_{AB} + k_{-A}} \approx \frac{F_A}{k_{-A}} \quad (5)$$

$$[B]_0 = \frac{k_{AB}[A]_0}{k_{BA} + k_{-B}} \quad (6)$$

It requires that B has a finite turnover with $k_{-B} >> k_{BA}$ because the equilibrium between A and B demands $k_{BA} << k_{AB}$. Moreover, k_{AB} must be very small, i.e. $k_{AB} << k_{-B}$ and with $k_{-B} < k_{-A}$ (B being less decomposable than A): $k_{AB} << k_{-A}$. Hence, we have the normal situation where A is determined solely by its metabolism $[A]_0 = F_A/k_{-A}$ and the concentration of B is practically zero.

However: How small is "zero"?

Before I come back to an answer to this question let us look at the initial condition in case of an infection, i.e. in case of an "external" employment of B.

It may be surprising to realize that the solution we obtain is not dependent on the concentration $[B]_0$. The "normal" steady state discussed above is solely a consequence of the relative magnitudes of the rate constants. This can be seen most clearly from Eq. (4). If the autocatalytic rate term at the highest possible concentration of A, i.e. $[A]_0$ according to Eq. (5), is larger than $(k_{BA}+k_{-B})$ then, in order to keep [B] non-negative, [A] must be depleted and drop to a value considerably smaller than $[A]_0$. Correspondingly [B] would rise to larger values (in accordance with a small denominator in Eq. (4)). In this case the second steady-state solution (Appendix A) would hold, yielding (with $K_M >> [A]_0$):

$$[B]_\infty \approx \frac{F_A}{k_{-B}}\left\{1 - \frac{k_{-A}\,k_{-B}}{F_A\,k_T/K_M}\right\} \approx \frac{F_A}{k_{-B}} \quad (7)$$

$$[A]_\infty \approx \frac{k_{BA} + k_{-B}}{k_T/K_M} << [B]_\infty \quad (8)$$

Eqs. (7) and (8) say that A is almost completely turned over to B which even accumulates because $k_{-A} > k_{-B}$. The index ∞ has been chosen in order to indicate that this is the "final" stable state the system could reach under certain conditions. The approximations in Eqs. (7) and (8) refer to the assumptions above regarding the magnitudes of k_{AB}, k_{-A}, k_{BA}, k_{-B} and F_A. However, the principal conclusion does not depend strongly on these assumptions. It reads: if the Prusiner mechanism could ever become effective, it must refer to this second steady-state solution, which

is solely dependent on the relative magnitude of the rate constant of the autocatalytic term with respect to the metabolic terms. In other words, the "normal" state in terms of the (linear autocatalytic) Prusiner mechanism is not only of a non-equilibrium, but also of a non-steady-state nature. This means that the reactions are too slow to reach the steady state (denoted by the index ∞) which is always the fatally pathogenic state. It is important to realize that this situation is entirely congruent with Prusiner's assumptions. The first steady state (Eqs. (5) and (6)) does not represent the normal situation in the absence of infection. It is the second steady-state solution (according to Eqs. (7) and (8)) that applies, regardless whether infection took place or not. Infection only accelerates a process which is otherwise too slow to become fatal during our normal lifetime. This aspect will certainly be of interest when observing other neuro-degenerative processes, such as those present in Alzheimer's disease. Now we must answer our question: "How small" is meant by "zero"?

A zero concentration of B requires that it be formed "sufficiently" slowly — despite the fact that its free energy favors B. What can we call "sufficiently slowly"? Let us assume a first order reaction by which just a single molecule of A (per liter) is transformed to B within the period of one year, indeed a very slow reaction. With $[A]_0$ being as small as nanomolar we have 10^{15} molecules of A per liter. Whatever the true value of $[A]_0$ is, this compound, which is the natural host protein is present in a huge number of single molecular copies — this is a simple consequence of the large size of Avogadro's number (i.e. about 10^{24}). In order to transform only one molecule of A into B (per liter and year) the rate constant k_{AB} must not be larger than 10^{-23} to 10^{-22} s^{-1}. For a linear autocatalytic mechanism one copy suffices to start an exponential "avalanche". This situation is well known from Darwinian population dynamics where in a large population just one (advantageous) mutant copy may appear, grow up and soon dominate the whole population. Even with a catalytic term $\left(\frac{k_T[A]}{K_M + [A]}\right)$ [1] as small as one per liter per year (meaning about one doubling every year) the B-population would amplify a single copy to the same size as the initial A-population (assumed to be nanomolar) within 34 to 35 years. (Note that the initial production of A via k_{AB} is linear, the autocatalytic amplification, however, exponential with time.) Of course, there is some threshold due to the metabolic decomposition of B which is supposed to be fairly small. However this may be, I am discussing here the particular case relevant to prion diseases where the autocatalytic term must be larger than the decomposition term. An autocatalytic doubling less than one per year is unrealistic anyway. It would mean that the progression of the disease would be correspondingly slow, also in case of an infection.

To make the long story short: "zero" concentration of B means a sub-threshold production of B of less than one copy within periods of years, i.e. $k_{AB} << 10^{-22}$ s^{-1}! Otherwise B will grow spontaneously even without infection. For this to happen (in particular in the case of infection) the autocatalytic term requires turnover numbers of at least one per year (and since $[A]/K_M<1$) $k_T >> 10^{-8}$ to 10^{-7} s^{-1}. Note that the turnover numbers of enzymatic reactions can be larger than 10^3 s^{-1}. So in order to work, the mechanism has to navigate between a "Skylla" of an extremely low value of k_{AB} and a "Charybdis" of a sufficiently high turnover number k_T, so that the ratio k_T/k_{AB} is large compared to 10^{15}. Such a catalytic enhancement (being expressed by this number) looks unrealistic to me, given a non-cooperative (linear) autocatalytic mechanism. I am not aware of any non-cooperative enzymatic turnover which realizes such a rate enhancement.

My conclusion is — as appealing as the idea of "induced fit" is — non-cooperative autocatalytic prion formation is quite unlikely. Logically there is nothing wrong with the principle of the Prusiner mechanism, but the conditions under which a linear autocatalytic mechanism could work seem to me too narrow and unrealistic.

4. What about a cooperative Prusiner mechanism?

Since Prusiner has never presented a mathematical analysis of the autocatalytic mechanism which he

[1] A realistic example would be: $[A] = 10^{-9}$ M; $K_M = 10^{-4}$ M, requiring a k_T of 10^{-3}–10^{-2} s^{-1}.

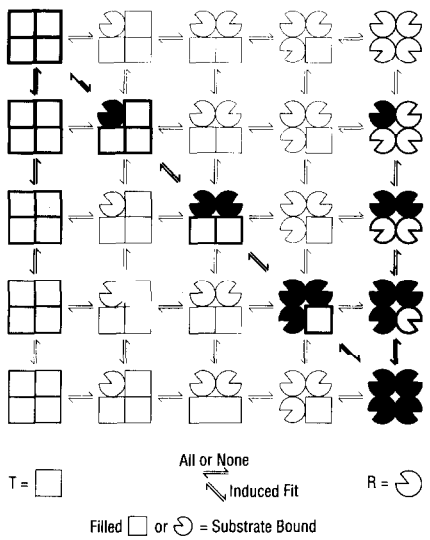

Fig. 2. A generalized model of allosteric enzyme action. The vertical line of states on the left side of the diagram are called T-states, those on the right side R-states. Binding of substrate favors the R-states. Two limiting cases of cooperative binding are represented by "all or none" transitions between T- and R-states [21], or an "induced fit" (diagonal) according to Ref. [20].

has proposed from his excellent experimental work, I would not hesitate to grant him credit for any type of autocatalytic mechanism of prion diseases.

The idea of "induced fit" played an important role in the kinetics of allosteric enzymes where it was successfully applied by Koshland and his school [20]. Fig. 2 shows a general reaction scheme of a four-subunit allosteric enzyme, in which the Monod–Wyman–Changeux [21] and the Koshland mechanism appear as two straightforward limiting cases. This general scheme was published first in 1966 [22] and has been re-invented several times since then [23].

Monod, Wyman and Changeux applied the idea of symmetry conservation to conformational changes of enzymes consisting of several subunits. Symmetry conservation implies that all subunits exist in either of two conformations (called R- and T-state), between which they switch cooperatively in an all-or-none fashion, depending on the substrate binding. Since one of the two states has a higher substrate affinity catalytic turnover can be regulated depending on the availability of substrate. Hemoglobin (though not an enzyme) is a famous example [24]. In the lung its affinity for oxygen is so high that it becomes saturated with oxygen, whereas oxygen is easily given off at the sites of respiration where the dissociation is triggered by a conformational change in the low oxygen level environment. I remember long discussions with Jacques Monod about the necessity of symmetry and on the question whether symmetry in biology is an "a priori requirement" or an "a posteriori" result due to selection of a certain advantage [25]. Jacques clearly was a Platonist, while I pleaded for the "a-posteriori" case. He was extremely pleased when our first experimental results on the fast kinetics of glyceraldehyde phosphate dehydrogenase (GAPDH) matched exactly his theory. Kaspar Kirschner [26] in our laboratory found three relaxation times with this enzyme, one related to substrate binding to the R-state, another one to substrate binding to the T-state, and a third one demonstrating the co-operative conformational change between R- and T-states. Later studies with other enzymes have also verified the presence of subsequent conformational changes that are associated with an increased binding affinity of substrate, typical for the "induced fit" mechanism proposed by Koshland (diagonal in Fig. 2).

I chose this lengthy introduction to demonstrate the close relationship between "induced fit" and cooperativity. The cooperative mechanism of prion formation (Fig. 3) follows closely Prusiner's ideas, but it is only one of several possible alternatives. If the two-dimensional representation in Fig. 3 would resemble the actual interactions, we would have to assume a certain (smaller) catalytic effect due to the

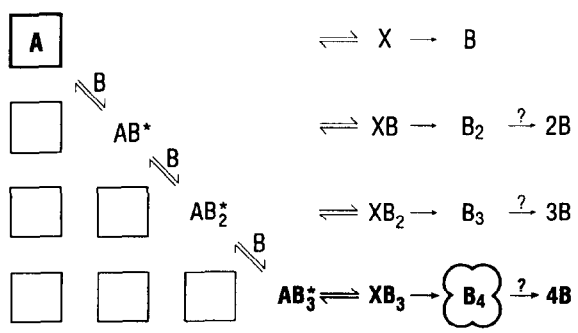

Fig. 3. Model of a cooperative Prusiner mechanism of autocatalysis.

(one-face) interaction between a circle and a square, which would also apply between dimer and trimer. A comparatively large cooperative effect would only be expected for the (two-face) interaction between trimer and tetramer. More realistically, there would be a monotonic increase of "induced fit" towards a tetrahedral structure in three-dimensional space, and this could apply to a tetramer or any higher i-mer.

A general treatment of protein aggregation that applies particularly to the problems treated in the next section, is given in Appendices B and C. I therefore restrict myself here to a more qualitative discussion considering the changes that are relevant in comparison to the linear model discussed in the preceding section.

The cooperative model retains an essential prerequisite from the linear model, namely that state B is favored versus state A on the basis of their free energies. This guarantees the turnover from A to B wherever the conditions are favorable. There is a certain vagueness with respect to this condition because B now exists in different states of free energy corresponding to free B or to any of the states of aggregation. I shall come back to this point in the next paragraph where it becomes of obvious importance.

In this model in which the thermodynamic equilibrium also favors state B we have to assume, as before, that the non-catalytic production of B is slow enough, so that the concentration of B cannot rise to any appreciable level in the absence of infection. In addition, we must assume that linear catalysis is slow enough so that it cannot successfully compete with metabolic removal. Only higher order catalysis can do so, provided that the level of [B] is high enough. In other words, cooperativity can introduce a threshold for [B] at which the system switches from one steady-state condition to the other. This is most easily seen if we consider the case where only in the last step, i.e. between trimer and tetramer in Fig. 3, the catalytic term becomes effective.

Let us assume that only the cooperative complex is catalytically active:

$$iA \underset{k_{BA}}{\overset{k_{AB}}{\rightleftharpoons}} (i-1)B + A \overset{K_{i-1}}{\rightleftharpoons} B_{i-1} + A \overset{K'_M}{\rightleftharpoons} B_{i-1}A \xrightarrow{k_\tau} B_i \xrightarrow{?} iB \qquad (9)$$

In addition we have "metabolic buffering" of A

$$\xrightarrow{F_A} A \xrightarrow{k_{-A}}$$

and also metabolic decay of B (k_{-B}). (For the question mark in the last step of Eq. (9) cf. Section 6.)

The system will, of course, pass through several intermediates which may be stationary (as treated in Section 5). For i=2 an autocatalytic term of this kind is present in the well known "Brusselator" model of Prigogine and Lefever (cf. Appendix B) where it causes bifurcation (i.e. transition from a focal fixed point to limit cycle oscillation).

In the case of cooperativity with i=2 Eq. (4) changes to:

$$[B]_s = \frac{k_{AB}[A_s]}{k_{BA} + k_{-B} - \dfrac{K_1 k_\tau [A]_s}{K'_M + [A]_s}} [B]_s \qquad (10)$$

where K_1 is an equilibrium (or steady-state) constant for the dimerization of B and K'_M a correspondingly adapted Michaelis constant for the reaction with A.

As long as the catalytic term is small compared to $(k_{BA} + k_{-B})$ we get the first steady state mentioned in the preceding section; this supposes that $[B]_0 = \dfrac{k_{AB}[A]_0}{k_{BA} + k_{-B}}$ does not reach the threshold value defined by

$$k_{BA} + k_{-B} = \frac{K_1 k_\tau [A]_0}{K'_M + [A]_0} [B]_{thr} \qquad (11)$$

Whenever the threshold is passed, e.g. by infection, the system switches over to another stable steady state in which B prevails. In order for the cooperative mechanism to work, the constants must be of such a magnitude that $[B]_{thr}$ can lie between $[A]_0$ and $[B_0]$.

By adding cooperativity to the "induced fit" mechanism of Prusiner, his hypothesis can work over a wider and more meaningful range of rate constants. A serious limitation is still the necessity to suppress linear autocatalysis. This is a necessary prerequisite as long as the equilibrium favors the state B relative to the state A (cf. the comparative discussion in Section 6).

5. The Lansbury mechanism of "plaque formation"

Lansbury Jr. [27–31] proposed a mechanism of pathogenic prion formation which, at a first glance, shows similarities to a generalized cooperative catalytic model, like the one presented in the preceding section. However, it involves three major modifications:

1st The Lansbury mechanism does not "explicitly" require catalysis.

2nd The natural state in a host cell, denoted by "A", can be the thermodynamically preferred state present in a (possibly rapidly established) equilibrium A ↔ B.

3rd Free B-particles — even under non-pathological conditions — are in a state of supersaturation with respect to aggregation or crystallization. However, in the absence of nuclei they may be indefinitely metastable, whereas when nuclei are present (through prion infection) they begin to aggregate in a (possibly) unlimited manner.

The latter condition actually invalidates the assumption of a true equilibrium between A and B. The prionic form is represented by an aggregate of B; the monomeric B state as well as the (initial) A state, appear only as metastable intermediates. In a similar fashion the third condition also formally abandons the first assumption. The nucleus may be considered as a "passive" autocatalyst for the transformation of A into the prionic state. Moreover, such a process is virtually indistinguishable from any "active" form of catalysis, i.e. a direct interaction of A-particles with some "catalytic surface" of the B-aggregates. Such an effect was mentioned by King [32] in a discussion of a paper by Caspar [33] on self-control in the construction of the protein sheets of icosahedral viruses.

Nucleation phenomena as involved in droplet formation in supercooled vapors as well as in precipitation and crystallization of solids from supersaturated solutions were fashioned by physical chemists — experimentally and theoretically — during the first half of this century. (cf. the works of Volmer [34–36], Frenkel [37], and Becker and Döring [38]). Lansbury [28,30] stresses the formation of one-dimensional crystals and their folding up into nuclei that enhance cooperative aggregation. Those "linear crystals" may not show true phase transitions. Their discrete chemical aggregation mechanisms are most adequately treated by models that consider the individual molecular reaction steps. This reminds me of a period in the early sixties when we tried to understand the cooperative nature of helix-coil transitions in both proteins and nucleic acids, as well as the formation of the double-stranded duplex between complementary oligonucleotides. [39,40] Work done at Göttingen and at Yale brought about a theoretical understanding of those cooperative phenomena (cf. Saunders and Ross [41], Schwarz [42], Crothers et al. [43]). Similar ideas apply to the cooperative association of proteins such as insulin, trypsin and hemoglobin (Chotia and Janin [41], or the process of actin filament formation (Wegener and Engel [45]), or virus coat formation as already quoted above.

A particularly striking example of cooperativity is the aggregation (gelation) of deoxyhemoglobin-S as observed in sickle cell anemia. A very sharp transition within extremely narrow ranges of concentration and temperature was observed and shown to involve the formation of a nucleus comprising as many as thirty protein molecules (Hofrichter et al. [46]).

Such a nucleus formation should not be envisaged as one singular "high order" reaction. If n particles are required to form a stable nucleus, these n particles do not simply encounter each other in one multimolecular nth-order process. Such would happen far too rarely to be of any relevance for our considerations. When we refer to the kinetic barrier presented by the formation of a nucleus, we mean rather a multiple-step process of aggregation. Below the critical concentration the aggregation is slowed down by the reverse reactions, i.e. by the dissociation of the intermediates that are formed. Hence the apparent nth-order reaction is broken down into a sequence of largely reversible second-order association processes which eventually may — or may not — establish the critical nucleus. Only after the formation of (or seeding with) such a critical nucleus is (further) aggregation favored over dissociation. The actual "apparent" order of the overall process depends to a great extent on the kinetic details of the interconnections between the many individual reaction steps, where certain reaction steps may be rate limiting.

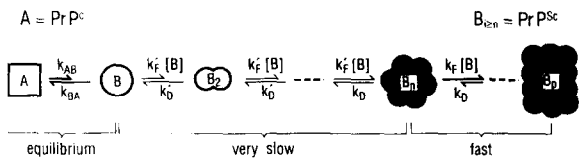

Fig. 4. The Lansbury mechanism of nucleated plaque formation (see text).

Fig. 4 represents the Lansbury mechanism in an abstract form corresponding to a cartoon shown in his Fig. 1, Ref. [30]. The notation follows the definitions given above: A is the normal host form in its preferred conformation. Metabolism is supposed to provide a constant level of A; B is the non-pathogenic monomeric protein having a pre-prion conformation. $B_2....B_i B_j....B_n....B_p.... = 2$-, i-, j-, n- or p-fold aggregates of B. Index n indicates the minimal nucleus size required for the indefinite progression of aggregate formation when $p > n$.

Rate constants		Rate terms
Non-catalytic conversion of A to B	k_{AB}	$k_{AB}[A]$
Non-catalytic conversion of B to A	k_{BA}	$k_{BA}[B]$
Formation of B_j from B_i	k_{ij}	$k_{ij}[B][B_i]$
Dissociation of B from B_j	k_{ji}	$k_{ji}[B_j]$

In a simplified model we may define two classes of formation and two classes of dissociation rate constants:

Prenuclear, i.e. $i < n$: $k_{ij} = k'_F$; $k_{ji} = k'_D$
Postnuclear, i.e. $i \geq n$: $k_{ij} = k_F$; $k_{ji} = k_D$

Since k_{ij} always associates with B, we define the dimensionless ratio

$$\frac{k_F[B]}{k_D} = q > 1 \quad \text{and} \quad \frac{k'_F[B]}{k'_D} = q' = \sigma q < 1 \qquad (12)$$

For a kinetic treatment the reaction scheme in Fig. 4 requires as many rate equations as there are specified states (i.e. $p + 1$). The solutions of these coupled non-linear differential equations cannot be given in a closed analytical form. If we knew all individual rate constants, we could carry out a numerical integration on a computer; we would do this if our objective were a comparison with or adaption (of parameters) to experimental results. The aim of this paper is not a quantitative *description* of real data, but rather an *understanding* of the principles that control reality. The model chosen must be simple enough to yield lucid results. On the other hand, it must be complex enough to comprise the essential influences that characterize nucleated aggregation.

First, let me describe the simplified version in words before turning to equations. We assume with Lansbury that A is the dominant host state. To a minor extent, A can transform into the pre-prion state B, which in its monomeric form is not pathogenic. If the turnover between A and B is fast enough (where k_{BA} is larger than k_{AB}), A has a "buffering" effect on B as well as on all subsequent states of aggregation down to B_n, the state that characterizes the nucleus. "Fast enough" means fast in comparison to any net reaction flow from B down to B_n. I say "down" because of the second assumption, namely: $q' < 1$; this inequality means that for any aggregation step between B and B_n the (chemical) forward flux rate parameter, $k_F'[B]$, is smaller than the reverse rate parameter of this step, k_D' (both given in reciprocal seconds). In this case the concentrations of the aggregated states B_i steadily decrease from B through B_2 etc., "down" to B_n. At B_n the situation — as far as this model goes — changes abruptly. Now the forward rate becomes larger than the reverse rate, depleting B_n quickly in favor of B_p with $p > n$ if it happens to be populated to any appreciable extent. It may be present on the average with less than one particle, showing up only sporadically in a stochastic fashion. The total net flow of reaction must pass this bottleneck, and the least populated state determines the narrowness of the bottleneck. How sharply the concentrations of prenuclear aggregation states drop depends on the size of the cooperativity parameter σ, which in reality may change on the pathway from B_2 to B_n.

In Appendix C (in which the mathematical details and a comparison with previous models can be found) it is shown for realistic σ values that the sum of concentrations of all prenuclear states of aggregation, or better, the amount of B contained in all these

states, i.e. $\sum_{i=2}^{n} i[B_i]$, will be quite small in comparison to [B] and in particular to [A]. This is a situation to which an assumption of steady state applies for all prenuclear ($i < n$) states.

Remember that the steady-state assumption is inherent to the Michaelis–Menten mechanisms of enzymatic turnover, which is based on very similar premises. An enzyme, according to the classical definition of a catalyst, enhances a reaction without influencing the equilibrium states of substrate and product. This is only possible if the enzyme is present at a sufficiently low concentration compared to substrates and products, which certainly is not always true when enzymes function under natural conditions. With $[S] >> [E_0] = \sum_i [E_i]$, where S refers to substrate and E_i to any enzyme-coupled state, we have

$$\frac{d[S]}{dt} >> \frac{d\sum_i E_i}{dt} \approx 0$$

which is the basic assumption for the derivation of the Michaelis–Menten expression.

By the same token we can make this assumption for our bottle-necked nucleation reaction as long as q' is sufficiently small. For any pre-nuclear state of aggregation (cf. Appendix C) we obtain:

$$\frac{[B_i]}{[B_{i-1}]} \approx \sigma q \quad \text{or} \quad \frac{[B_i]}{[B]} \approx (\sigma q)^{i-1}$$

for $\sigma q << 1$: $[B_i]/[B] << 1$ decreasing with increasing i. The total amount of B contained in aggregates is:

$$\sum_{i=2}^{n} i[B_i] = [B] \cdot \sum_{i=2}^{n} i(\sigma q)^{i-1}$$

which is explicitly calculated in Appendix C.

For $\sigma q < 0.25$ this sum is already < [B] and hence << [A].

For the rate of nucleus formation we obtain:

$$\frac{d[B_n]}{dt} = k'_F[B_{n-1}][B] \approx k'_F(\sigma q)^{n-2}\,[B]^2 \tag{13}$$

The required buffering action of B by A is established with a rate (the rate constants being k_{AB}, k_{BA}) that is rapid compared to the leakage through the bottle-neck of the nucleus B_n. As we shall see, this condition is usually fulfilled even if the values of k_{AB} and k_{BA} "sound" pretty small. B may get depleted only in the case of infection requiring A → B to become rate limiting.

Each nucleus now incorporates — with a "rate constant" $k_F[B]$ — further B-particles leading to an increase of the total number of particles integrated in B_p ($p > n$), i.e. $\sum_{p\geq n} p[B_p]$, which proceeds (according to this model) with the square of the time:

$$d\sum_{p\geq n} p[B_p]/dt = k_F[B] \cdot \sum_{p\geq n} [B_p]$$

$$\sum_{p\geq n} [B_p] \sim \int^{t} \frac{d[B_n]}{dt}\,dt = k'_F(\sigma q)^{n-2}[B]^2\, t \tag{14}$$

$$\sum_{p\geq n} p[B_p] \sim k_F k'_F(\sigma q)^{n-2}[B]^3\,\frac{t^2}{2}$$

As a numerical example let us assume maximum (i.e. diffusion-controlled) rate constants k'_F and k_F of $10^7\ M^{-1}\ s^{-1}$, a ratio of k_{AB} to k_{BA} of 10^{-3}, and for σq a value of 10^{-2}. A nucleus consisting of six protein molecules (according to Lansbury this is the minimum size) would then form with a rate of about 2×10^6 nuclei per liter and year. They would (in a manner proportional to the square of the time) incorporate something like 10^8–10^9 B-particles per liter per year. After thirty years this turnover would approach the order of magnitude of B particles stationarily present which, however, has been assumed to be still three orders of magnitude below the level of A particles. This example is pure fiction, but it demonstrates quite well the explanatory power and limitations of the Lansbury model.

How realistic is the model?

The critical values are those of q, σ and n. The ratio q is a measure of supersaturation. Hence q has

to be larger than one. $q > 1$ means: a seed grows faster than it decays. On the other hand, q must not be too large because σq has to be sufficiently smaller than one. A uniform value of σ for all steps of aggregation up to the critical size is, of course, unrealistic. The uniform value can be considered a geometric mean of the individual values σ_i; e.g. for an aggregate of $i+1$ particles it is the ith root of the product of all individual σ-factors. For the critical size we have

$$\sigma = \left(\prod_{i=2}^{n-1} \sigma_i \right)^{\frac{1}{n-2}}$$

The individual σ values will increase monotonically from a (possibly) very low value for the dimer B_2 (i=2) to the critical size n above which they become equal to one. Hence the expression for the rate of nucleus formation can be described by one overall factor which is the product of all individual factors. In the above numerical example with $(\sigma q)^4 = 10^{-8}$, the supersaturation q could be 10 and $\sigma = 10^{-3} = (10^{-4} \cdot 10^{-3} \cdot 10^{-3} \cdot 10^{-2})^{1/4}$ or $(10^{-6} \cdot 10^{-2} \cdot 10^{-2} \cdot 10^{-2})^{1/4}$.

How realistic are those σ-values? Wegner and Engel [45] calculate from their data on the cooperative association of actin to actin filaments for $A + A \leftrightarrow A_2$ $K = 4 \times 10^{-2}$ M or $\Delta G = 2$ kcal mol^{-1} and for $A_n + A \leftrightarrow A_{n+1}$ $K = 1.7 \times 10^5$ M^{-1} or $\Delta G = -7$ kcal mol^{-1}. This corresponds to $\sigma_1 = 2.35 \times 10^{-6}$.

Below the critical size, σq must always be smaller than one (the definition of the critical size is when $\sigma_n q \approx 1$). This includes the possibility that σ_n is still smaller than one at the critical size n, depending on the concentration of B or A respectively. Otherwise we may imagine all sorts of "bottlenecks" with different shapes.

In conclusion, Lansbury's mechanism can work if special prerequisites are fulfilled. As in the case of a cooperative Prusiner mechanism the range of conditions is still fairly narrow. In the following paragraph I shall compare the pros and cons of the various mechanisms.

6. Which mechanism is the correct one?

Let me immediately specify this question further: is the mechanism likely to be non-catalytic, catalytic or even autocatalytically self-enhancing?

The linear Prusiner mechanism clearly is of the latter type yielding exponential amplification of prions whenever they appear on the "stage". We have discussed numerically (in Section 3) the stringent conditions required for this case to materialize. Spontaneous production of B (=PrP^{Sc}) must remain below its metabolic decay rate which itself is controlled by the level of [B]. The consequence is — apart from certain quite unrealistic conditions — either there is no infection at all, i.e. $\frac{d[B]}{dt}$ is negative, or a spontaneous outbreak of the disease occurs with exponential growth in every case.

The cooperative Prusiner mechanism is free of this difficulty. It has a threshold of B below which the growth rate (being very slow) can be controlled by metabolic decay; above this threshold, i.e. as a consequence of infection, growth inevitably breaks through. Such a mechanism still can be autocatalytic showing exponential growth.

Having analyzed the Lansbury mechanism we are justified to ask whether we need "active" catalysis at all? The answer is: we may not need it; but are we able to exclude it? If the crystal growth is favored by free energy, the surfaces of crystals may well exert catalytic power to compel the transformation of A(=PrP^c) to B_i which for $i > n$ is PrP^{Sc}. After all, these forces should be of the same nature as those active in a Prusiner mechanism.

We then face a question that is relevant to all the mechanisms discussed. If they are catalytic in this way, how can they also be (actively or passively) self-enhancing, i.e. "truly" autocatalytic with an exponential growth characteristic?

The Prusiner mechanism is autocatalytic in this sense only if either the dimer B_2 or the cooperative polymeric form B_i, that forces the transformation to B, dissociates rapidly; otherwise, the autocatalytic form becomes inactivated, or the dissociation becomes rate-limiting, preventing exponential growth. Likewise, in the Lansbury mechanism the crystals should be "ground" in order to allow for an autocatalytic increase of the number of seeds. Lansbury mentions this [30], but he does not tell us how it is to be done. Lansbury [30] writes: "In our model, this could be explained by the idea that the PrP^{Sc} aggregate can be fragmented to produce multiple seeds, each of which can be a template for further polymerization of host

PrP^c". He then describes experiments on fragmentation of amyloid fibrils by sonication, which indeed increases seeding efficiency [27,28]. However, who is the Maxwellian demon who carries out the sonication or any other kind of "grinding" in nature? What usually happens in crystallization is "Ostwald ripening" which favors larger crystals rather than smaller ones. Perhaps the "linear crystals" of Lansbury differ in this respect in being more fragile.

In the absence of reactivation of the catalyst any catalytic mechanisms would soon "saturate". The crystal formation would remain in a growth phase that is (nearly) quadratic in time. Perhaps the finding that prion infection has the very low efficiency of one infectious unit in 100 000 prion particles [5,47] hints that crystals grow large and remain so.

We may well ask whether we really need an exponential growth phase. Wouldn't it cause difficulties of the kind mentioned in Section 3? In crystal growth we still have many other possibilities, an almost complete repertoire of mechanisms between quadratic and exponential time characteristics. A crystal may grow only linearly, or it may branch out. In the first case the reactant concentration is $\sum_i B_i$, in the second something closer to $\sum_i iB_i$. Moreover, since plaque formation appears at the membrane interfaces, the whole mechanism may involve both solution and surface kinetics in a similar way as outlined by Adam and Delbrück [48] or by Goldman and Katchalsky [49]. These details can only be clarified if more experimental results become available.

The answer to the question posed in the headline of this section may be a Solomonic one, i.e. both are right. I cannot see how to distinguish active from passive catalysis, and I cannot see how to prevent active catalysis in either of the cooperative mechanisms. On the other hand, if cooperativity is based on aggregation, I cannot see how to stop crystal growth. Even in the Prusiner mechanism, crystallization may be a secondary outcome. The main difference then resides in the question: which of the two monomeric states is favored by equilibrium, A or B? That question can only be decided by suitable experiments. Whatever the answer, it would not invalidate the "protein only" hypothesis at all. Both, the Prusiner and the Lansbury mechanisms are prototypes, and in detail, they may have several subtypes. Mechanisms that require the fixation of the proteins to membranes, and thereby influence their reaction–diffusion coupling, should especially be considered.

Answers to these question may be crucial in specifying the true mechanism of infection. So far I have not discussed any possible causes of pathogenicity. What I have done was merely to compare mechanisms that have been proposed. If I understand Weissmann [5] correctly, then this question cannot yet be answered with certainty. Is it the depletion of A (=PrP^c) which causes some secondary deficiency, or does the production of B in any of the aggregated states trigger misbehavior? Does plaque formation itself destroy the nerve membrane, or is the cause something we have not yet thought of, for instance a substance present under *in vivo* conditions and so far not considered in experiments carried out *in vitro*. What is still missing is the verification of the disease under *in vitro* conditions [50].[2]

Whatever it is, similarities of prion diseases to other neurodegenerative diseases like Alzheimer's indicates that the cause at least is related to some basic mechanism of the sort I have discussed in this paper. If this is true, it might lead us to redefine the borderline between health and illness.

7. What conclusions can be drawn about diagnosis and treatment of the disease from "all this gesinta"? [3]

If a theory is worth anything, it should allow a sharper formulation of the questions and thereby lead to new experiments. Only experiments can tell us which solution Nature has chosen from the rich repertoire she has at her disposal. Experiments therefore cannot be replaced by theory; nevertheless, as

[2] The involvement of a substance other than protein, e.g. a lipid, may (verbally) invalidate the term "protein only" model.

[3] The term "gesinta" was explained to me by my colleague Manfred Schroeder, author of the best-selling book "Fractals, Chaos, Power Laws". It is New York slang. Mother asks her child coming from school: Did you do your gesinta alright? Gesinta derives from elementary calculus: one and one "goes into" two.

Einstein once pointed out to Heisenberg [51] "only theory can tell us what can be observed and how to interpret what we observe".

Experiments should aim at determinating the parameters appearing in the various models, such as the rate constants, q and σ values, as well as the nucleation lengths. Measurements could clarify whether active catalysis is present and what the nature of the reaction complexes is. The main question, namely whether aggregation of prions is a necessary and sufficient prerequisite of pathogenicity still remains unanswered [50]. The present paper clearly emphasizes that aggregation is necessarily involved in either of the mechanisms proposed.

However that may be, the involvement of aggregation at least offers a new access to sensitive diagnosis. We have recently shown how to use fluorescence fluctuation measurements for an extremely sensitive detection of the presence of molecular complexes. The technique is known as fluorescence correlation spectroscopy (FCS) and has been pioneered independently by Elson, Magde and Webb [52] in the US and by Rigler and his coworkers at Stockholm/Sweden [53]. The principle consists of focusing a laser beam into a very small volume element comprising only a fraction of a femtoliter (fl = 10^{-15} l). In a protein solution at picomolar (10^{-12} M) concentration a protein molecule will move into this volume element only every few seconds, where it will reside for a few milliseconds (depending on its diffusion coefficient). If the molecule is labeled with a fluorescent dye, bursts of fluorescence light quanta may be observed using an autocorrelation technique with time-resolved detection. (For a detailed description cf. Ref. [54].) The fluctuation of fluorescence light is detected using confocal optics. These fluctuations can be seen best when the concentration is below nanomolar, and when the focused spot is as small as mentioned. Our particular trick for making aggregates visible is to use two dyes with different fluorescence wavelengths and to record via cross-correlation solely the presence of both dyes in one and the same aggregate. This type of cross correlation has been studied in a dissertation recently carried out at our laboratory [55]. It should not only allow the detection of the presence of complexes, but also allow a determination of their size. Apart from *in vitro* measurements aimed at clarifying mechanisms, this technology is also suitable for routine diagnostic purposes [56]. Sensitive detection of aggregations should as well be of benefit for an early diagnosis of Alzheimer's disease. Since we are getting older and older this may be a true blessing.

Concluding remarks

This article is written in a style that makes it accessible to a large audience interested in the prion problem. The explanations given may, in some cases, be self-evident for physical chemists. However, it is my hope that they also reach biologists and stimulate them to look occasionally into this journal if they propose kinetic models for biological processes.

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Appendix A: Linear Autocatalysis

The analysis is based on Fig. 1, on the definitions and on Eqs. (1) and (2) given in the text. The catalytic term will usually be a second order term since Michaelis constants will hardly reach the magnitude of [A] which is likely not to be too far from nanomolar. If saturation were achieved, representing the upper limit of speed of autocatalytic turnover, we could deal with a system of linear differential equations:

$$\frac{d[A]}{dt} = F_A - k_{-A}[A] - k_{AB}[A] - k_T[B]$$

$$\frac{d[B]}{dt} = k_{AB}[A] + (k_T - k_{-B})[B] \qquad (A1)$$

Since [A] initially is much larger than [B] all initial changes would affect mostly B while [A]

nearly remains constant, i.e. $[A]_0$. We can proceed for this case in two ways. Either we substitute F_A by $F_A[A]/[A]_0$ (i.e. $[A] \approx [A]_0$) or we solve directly the second equation with $k_{AB}[A]_0$ being a constant term. In the latter case we obtain ($k_{AB} << k_{-A}$; $k_{BA} << k_{-B}$):

$$\frac{d[B]}{dt} = k_{AB}[A]_0 + (k_T - k_{-B})[B]$$

with the solution

$$[B]_t = \left\{\frac{k_{AB}[A]_0}{k_T - k_{-B}} + [B]_{t=0}\right\} e^{(k_T - k_{-B})t} - \frac{k_{AB}[A]_0}{k_T - k_{-B}} \quad (A2)$$

For $k_T > k_{-B}$ the initial state A_0 is unstable and changes exponentially with time. This solution holds only as long as $[A]_t \approx [A]_0$. A new steady state then will be approached.

In the first case we have to determine the time constants τ_m from the eigenvalues $\lambda_m = 1/\tau_m$. The characteristic equation reads:

$$\lambda_m^2 - T\lambda_m + \Delta = 0 \quad (A3)$$

where T is the trace of the matrix of rate coefficients and Δ its determinant. The solutions are:

$$\lambda_1 \approx \frac{k_{AB}\, k_{-B}}{k_T - k_{-B}}; \quad \lambda_2 = (k_T - k_{-B}) \quad (A4)$$

The first time constant is very small and refers to a minor correction of the metabolic term due to the small, but finite, k_{AB}. The second one indicates an instability for $k_T > k_{-B}$ resulting in an exponential amplification of B (as long as $[A] \approx [A]_0$).

The nonlinear equations with the complete catalytic term cannot be solved explicitly. However, we get explicit expressions for the steady-states:

Starting from the two implicit steady-state relations Eqs. (3) and (4) in the text we obtain a quadratic equation for A:

$$[A]_s = \frac{F_A k_{cat} + k_{-B}(k_{-A} + k_{AB})}{2k_{-A}k_{cat}} \left\{1 \pm \sqrt{1 - \frac{4F_A k_{cat} k_{-A} k_{-B}}{[F_A k_{cat} + k_{-B}\,(k_{AB} + k_{-A})]^2}}\right\} \quad (A5)$$

with $k_{cat} = \frac{k_T}{K_M}$ (i.e. assuming $[A]_s << K_M$)

The positive root (referring to $k_{-A}k_{-B} > F_A k_{cat}$) is:

$$[A]_{s1} = \frac{F_A}{k_{-A}}\left\{1 - k_{AB}\frac{k_{-B}}{k_{-A}k_{-B} - F_A k_{cat}}\right\} \approx \frac{F_A}{k_{-A}} \equiv [A]_0 \quad (A6)$$

(the approximation requiring $k_{AB}/k_{-A} << 1 - \frac{F_A k_{cat}}{k_{-A}k_{-B}}$).

The negative root refers to $k_{-A}k_{-B} < F_A k_{cat}$ and has to be determined with high accuracy since large terms compensate leaving small differences:

$$[A]_{s2} = \frac{k_{-B}}{k_{cat}}\left\{1 - \frac{k_{AB}}{k_{-A}}\,\frac{1}{1 - \frac{k_{cat}\,F_A}{k_{-A}k_{-B}}}\right\} \quad (A7)$$

For $F_A k_{cat} >> k_{-A}k_{-B}$ and $k_{-A} >> k_{AB}$ it becomes:

$$[A]_{s2} \approx \frac{k_{-B}}{k_{cat}}\left(1 - \frac{k_{AB}k_{-B}}{F_A k_{cat}}\right) \approx \frac{k_{-B}}{k_{cat}} << [A]_0$$

The associated B-term, according to Eq. (4) in the text, then reads:

$$[B]_{s2} = \frac{F_A}{k_{-B}}\left(1 - \frac{k_{AB}k_{-B}}{F_A k_{cat}}\right) \approx \frac{F_A}{k_{-B}} \quad (A8)$$

(i.e. in the limiting case $F_A k_{cat} >> k_{-A}k_{-B}$ which means $k_{cat}[A]_0 >> k_{-B}$).

In the implicit form of Eq. (4) $[B]_s$ is proportional to the exceedingly small rate constant k_{AB}, while $[B]_{s2}$ according to Eq. (A8) is large. $[B]_\infty$ may be even larger than $[A]_0$ since probably $k_{-A} > k_{-B}$. The whole production of A now is metabolised through B.

The solution for $[B]_{S1}$, associated with $[A]_{S1}$, shows directly the influence of the exceedingly small constant k_{AB}. In the limiting case of $k_{cat}[A]_0 << k_{-B}$ we have:

$$[B]_{s1} = \frac{k_{AB}[A]_0}{k_{-B} - k_{cat}[A]_0} \approx \frac{k_{AB}}{k_{-A}} \cdot \frac{F_A}{k_{-B}} \frac{1}{1 - \frac{F_A k_{cat}}{k_{-A}k_{-B}}} \quad (A9)$$

If metabolic decay cannot compensate autocatalytic production the state S_1 is not stable. The important fact is that this is not dependent on the concentration of the autocatalyst, it is solely a matter of relative rate constants (cf. Appendix B).

Appendix B: Cooperative Autocatalysis

Host State: A. Prion State B. Effective Catalyst: B_i (see Fig. 3). All states intermediate between B and B_i are assumed to be stationary, present at low concentration. The catalytic term then is formally treated as:

$$iB + A \rightarrow (i+1)B \tag{B1}$$

Prigogine and Lefever [56] make an analogous assumption in their Brusselator model (i=2). For the results obtained this assumption is essential (cf. Section 6).

Rate Equations:

$$\frac{d[A]}{dt} = F_A - (k_{AB} + k_{-A})[A] - k'_{cat}[B]^i [A]$$

$$\frac{d[B]}{dt} = k_{AB}[A] - (k_{BA} + k_{-B})[B] + k'_{cat}[B]^i [A] \tag{B2}$$

The rate parameters are defined in Section 3.

These non-linear equations cannot be solved in explicit form which is also true for the steady-state equations (if $i \geq 2$). Implicit steady-state solutions read:

$$[A]_s = \frac{F_A}{k_{-A} + k'_{cat}[B]_s^i}$$

$$[B]_s = \frac{k_{AB}F_A}{k_{-A}k_{-B} + (k_{-B}[B]_s - F_A)k'_{cat}[B]_s^{i-1}} \tag{B3}$$

In his excellent textbook Nicolis [57] describes how to analyze for stability of solutions. One expands the rate equations around the steady-state and linearizes. The nature of the solutions can be derived from three numbers: the trace (T) and the determinant (Δ) of the matrix of linearized rate equations and the discriminant ($D = T^2 - 4\Delta$) of the root of the characteristic equation that determines the eigen values:

$$\lambda^2 - T\lambda + \Delta = 0$$

For $D > 0$ the two eigen values are real. Instabilities occur if at least one of the eigen values is positive, which requires $\Delta < 0$. For $\Delta > 0$ and $T > 0$ both eigen values are positive.

For comparison the Brusselator yields a stable focus below critical concentrations of the reactants with a bifurcation to an unstable focus and a limit cycle oscillation. Our model of cooperative catalysis also yields an instability at a critical concentration of B. At low concentration we have a stable focus yielding for $i = 2$

$$[A]_s = \frac{F_A}{k_{-A}}\ ;\ [B]_s \approx \frac{F_A k_{AB}}{k_{-A}k_{-B}} << [A]_s \tag{B4}$$

External supply of B (in the case of an infection) destabilises this solution and the system approaches a new stable focus with:

$$[A]_s \approx \frac{k_{-B}^2}{F_A k'_{cat}}\ ;\ [B]_s \approx \frac{F_A}{k_{-B}} - \frac{k_{-A}k_{-B}}{k'_{cat}F_A} \approx \frac{F_A}{k_{-B}} >> [A]_s \tag{B5}$$

With this approximation of B_s the denominator in Eq. (B3) would be zero, which actually means: exceedingly small, thereby compensating the exceedingly small value of k_{AB}. For the general cooperative case the catalytic term is to be replaced by

$$\sum_{i=1}^{n} k'_{cat,i}[B]_s^i [A]_s$$

Stability analysis uncovers a number of other interesting facts which, however, lie outside the scope of the present paper and will be presented elsewhere.

Appendix C: Kinetics of Plaque Formation

The mechanism is depicted in Fig. 4. We ask for the rate of nucleus formation. The steady-state assumption starts from the premise that the difference

of forward and reverse rate at each stage of the reaction is the same. If we denote these rates as

$$\begin{aligned} R_{01}&: \quad k_{AB}[A] - k_{BA}[B] \\ R_{12}&: \quad k'_F[B]^2 - k'_D[B_2] \\ R_{23}&: \quad k'_F[B][B_2] - k'_D[B_3] \\ &\vdots \qquad\qquad \vdots \\ R_{i,i+1}&: \quad k'_F[B][B_i] - k'_D[B_{i+1}] \end{aligned} \tag{C1}$$

The steady-state condition requires: $R \equiv R_{01} = R_{12} = R_{23} \ldots R_{i,i+1}$. If the rate $R_{i,i+1}$ for any $i \geq 2$ is multiplied with a factor $(k'_D/k'_R[B])^{i-1}$ we realize that the second and negative term in each of the Eqs. (C1) — except for R_{01} — will equal the first and positive term of the subsequent equation. Hence in summing up all equations from R_{12} to $R_{n-1,n}$ (where n characterizes the size of the nucleus) we are left with only the first and the last term:

$$\begin{aligned} R(n) \sum_{i=1}^{n-1} (k'_D/k'_F[B]^{i-1} \\ = k'_F[B]^2 - k'_D(k_D/k_R[B])^{n-1}[B_n] \end{aligned} \tag{C2}$$

or

$$R(n) = \frac{[k'_F[B]^2 \cdot (\sigma q)^{n-2} - k_D[B_n]]\,(1-\sigma q)}{1-(\sigma q)^{n-1}} \tag{C3}$$

if the reciprocal of $k'_D/k'_F[B]$ is called σq. This procedure [41,43] was applied by Hofrichter et al. [46] to yield the following expression for the rate of nucleus formation, which reads in the present notation:

$$R(n) = \frac{[B]^{n-1}k'^{n-2}_F\sigma(q-1)(1-\sigma q)}{k'^{n-3}_D(1-\sigma)} \tag{C4}$$

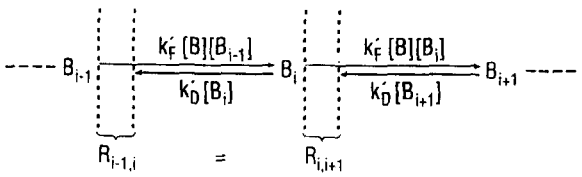
Supposing $q >> 1$, but $\sigma q << 1$ (i.e. $\sigma << 1$) Eq. (C4) reduces to

$$R(n) = k'_F[B]^2\,(\sigma q)^{n-2} \tag{C5}$$

which is identical with Eq. (13) in the text.

The first step in the reaction characterized by the rate R_{01} is assumed to be equilibrated, so that [B] always can be substituted by $\frac{k_{AB}}{k_{BA}}[A]$.

How well is the steady state assumption justified? Let us look more precisely at the reaction flow diagram:



If the "leakage" flow rate $R(i)$ is constant the steady state concentrations must decrease with increasing i, while a finite and positive R requires:

$$\begin{aligned} &k'_F[B][B_{i-1}] > k'_D[B_i] \\ &\frac{[B_i]}{[B_{i-1}]} < \frac{k'_F[B]}{k'_D} = \sigma q \end{aligned} \tag{C7}$$

For $\sigma q << 1$ the ratio $\frac{[B_i]}{[B_{i-1}]}$ indeed comes close to σq. Let us look at the smallest B_i for which such a flow diagram holds. It is B_{n+1} where n stands for "nucleus" meaning that at B_n k'_D changes to k_D and $k'_F[B]$ to $k_F[B]$ the ratio $\frac{k_F[B]}{k_D} = q$ being σ times larger than $\frac{k'_F[B]}{k'_D}$. Beyond this bottleneck there is no steady-state whatsoever.

According to the flow diagram we have

$$[B_i] = \frac{k'_F[B][B_{i-1}] + k'_D[B_{i+1}]}{k'_F[B] + k'_D} \tag{C8}$$

We know from Eq. (C7) that $\sigma q > \frac{[B_{i+1}]}{[B_i]}$. Let us assume

$$\frac{[B_{i+1}]}{[B_i]} = \sigma q(1-\varepsilon) \tag{C9}$$

Inserting it into Eq. (C8) yields

$$\frac{[B_i]}{[B_{i-1}]} = \frac{\sigma q}{1 + \sigma q \varepsilon} \approx \sigma q(1 - \sigma q \varepsilon) \qquad (C10)$$

Below the nucleation size ($i < n$) we find that all ratios $\frac{[B_i]}{[B_{i-1}]}$ are very close to σq with a maximum deviation at the "bottleneck", i.e. for $i = n - 1$, of $(1 - \sigma q)$ where $\sigma q << 1$. Since each aggregate B_i contains i B-particles the total amount of B contained in all aggregates below the nucleation length is:

$$\sum_{i=1}^{n} i[B_i] \approx [B] \sum_{i=2}^{n} i(\sigma q)^{i-1} \qquad (C11)$$

$$= [B] \left\{ \frac{1 + (\sigma q)^{n-1} \left[n(\sigma q)^2 - (n+1)\sigma q \right]}{(1 - \sigma q)^2} - 1 \right\}$$

Examples: $\sigma q = 10^{-1}$: $\sum_{i=2}^{n} i[B_i] = 0.234[B]$

$n = 10$

$\sigma q = 10^{-2}$: $\sum_{i=2}^{n} i[B_i] = 0.02[B]$

This sum is constant only up to the nucleation size. It becomes a function of time beyond that size, where all kinds of states B_p with $p \geq n$ can eventually be filled up. If nuclei grow without splitting up or branching out, $\sum_{p \geq n} p[B_p]$ will become a quadratic function of time.

The rate of nucleus formation is represented by Eq. (13) in the text (see also Eq. (C5)). It assumes that after the nucleus B_n has formed there is a negligible back flow $k_D[B_n]$. A possible correction term, assuming $\sigma << 1$ leaving $q > 1$ and $\sigma q < 1$ (like in Eq. (C4)), would be $(1 - \sigma q) \frac{q - 1}{q}$ to be multiplied with the rate expression Eq. (C5). However, this correction term must remain close to one because otherwise it may invalidate the assumption of a steady state (e.g. the term $\sigma = 1$ in the denominator of Eq. (C4) would produce a singularity).

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