

Some considerations on the kinetics of pathogenic prions formation

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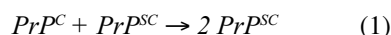
Summary. - Literature data, respective to kinetics of infectivities due to prion diseases, have been here reconsidered. The autocatalytic phenomenon of prion replication and the process of advancement of the infection are also considered. A model describing the interconversion from "normal" into "pathogenic" prions, and the subsequent growth of the infection, is proposed. This model takes into account the existence of two different steps: the first, slower, in which the interaction between the two different prions, with the transformation of normal prions into the pathogen ones, takes place. The second one, very fast, in which the degree of advancement of the infection assumes the form of an irreversible, rapid trend.

Key words: prion diseases, infectivity, kinetics, brain.

Riassunto (*Alcune considerazioni sulla cinetica di formazione di prioni patogeni*). - Dati riportati in letteratura e relativi a cinetiche di infettività per patologie prioniche sono stati riconsiderati secondo un nuovo approccio. Sono discussi il processo autocatalitico, che è alla base della replicazione prionica, ed il processo di propagazione dell'infezione. Su questa base è proposto un modello cinetico che descrive l'interconversione, in due stadi, dei prioni dalla forma "normale" a quella "patogena" ed il conseguente sviluppo dell'infettività. Tale modello considera l'esistenza di due differenti fasi: la prima, più lenta, in cui si verifica l'interazione tra due diversi prioni, la seconda, molto più rapida, in cui il grado di avanzamento dell'infezione assume l'andamento tipico di un processo rapido con elevato grado di irreversibilità.

Parole chiave: patologie prioniche, infettività, cinetiche, cervello.

Increasing interest has recently been devoted to the study of transmissible spongiform encephalopathies (TSE) characterized by prion infections that culminate in fatal neurodegenerative disorders due to deposit of protein aggregates in the brain. According to Prusiner *et al.* [1-7] the mechanism of prion replication involves "normal" prions (cellular prion proteins, PrP^C) and "pathogenic" prions (PrP^{SC}). The disease is due to the propagation in the organism of the pathogenic conformer, which originates, by an autocatalytic mechanism, from the PrP^C refolding. It is indeed the interaction between these two molecular entities that brings to a conformational change of the normal cellular prions that are converted into the pathological ones (reaction 1); while the molecular weight and the amino-acidic sequence are the same in both cases and are unaffected.



PrP^C is therefore necessary for both the development of the disease and the propagation of the pathogenic prions in the inoculated animals [8]. Although it has recently been demonstrated that the transition between the two prion conformers can occur reversibly in strongly denaturing media [9], the transition from the cellular to the pathogenic conformer, as well as the degree of advancement of the infection in the central nervous system are, both on thermodynamical basis [10] and from a pathological point of view, irreversible events.

More specifically:

a) the formation of pathogenic prions from cellular prions is an autocatalytic and cooperative process [1, 11], different from other controlled biological replication

processes belonging to the type of inherent autocatalysis. For reaction (1) it seems, therefore, sound to assume valid a here proposed reaction kinetic model;

b) the degree of advancement of the infection, and the consequent injury in the brain, takes place after the pathogenic prions are transported from the blood stream into the central nervous system. According to compartmental analysis, such an event can be described by a mono-compartmental model, i.e. representing the transport of prions from the blood stream to the central nervous system as a strictly irreversible process.

It follows that, by considering the general model (of a sort of autocatalysis and cooperative autocatalysis) proposed by Prusiner [1], in which two identical molecular species are involved as far as molecular mass, aminoacidic sequence, and secondary structure is concerned, one can write a schematic kinetic interaction sequence in the form: $A' + A \rightarrow [A'A] \rightarrow 2A \rightarrow \vartheta$, where, according to reaction (1), A' , A , and ϑ indicate respectively Pr^{PC} , Pr^{PSC} and the pathological effect. By starting from a general principle of the chemical kinetic, one can write that

$$-\frac{dc_{A'}}{dt} = kc_A^2 \quad (2)$$

$$-\frac{dc_{A'}}{c_A^2} = kdt \quad (3)$$

and by integration:

$$\int \frac{dc_{A'}}{c_A^2} = -\int kdt \quad (4)$$

$$-\frac{1}{c_A} = -kt + cost \quad (5)$$

This kinetic approach, however, is not operative since the punctual values of k and c_A are not detectable, while the pathological effect of the infection is usually referred to the unitary mass (generally one gram) of brain tissue.

We have then used experimental literature data, published in the past by different research groups in different laboratories, referring to LD_{50}/g of hamster 263K brain tissue. These results have already been discussed [12], but no decisive conclusions were drawn: interestingly, it has to be pointed out that, even if kinetic approaches have been presented to study the replication rate of pathogenic prions [13], no valid experimental kinetic models have, up to date, been considered.

By comparing a degree of advancement of a chemical reaction with a degree of advancement of the infection, several informations can be obtained. So that by indicating with ξ^* the initial value of the degree of

advancement of the infection, it is possible to expand the velocity of propagation of the infection, v_ξ , according to a Taylor's series, in the range around a given value ξ^*

$$v_\xi = v_{\xi-\xi^*} + \left(\frac{dv_{\xi}}{d\xi} \right)_{\xi-\xi^*} (\xi - \xi^*) \quad (6)$$

By limiting the expansion to the second term, and being

$$v_{\xi-\xi^*} = 0, \quad (7)$$

one has

$$v_\xi = (dv_\xi/d\xi)(\xi-\xi^*) = (d\xi/dt)I/V \quad (8)$$

and

$$v_\xi = (d\xi/V)/dt ; v_\xi = (dc_\xi/dt) \quad (9)$$

where (dc_ξ/dt) should be correlated to the value of the rate of pathogenic prions growth in the considered tissue. However at present time it is impossible to measure the concentration of prions in brain tissues and/or to calculate the kinetic constants respective to prionic interactions, while it is merely possible to evaluate the LD_{50} and hybrid constants. Owing these evidences and limitations and starting by equation (5), we find that by expressing the pathogenic prion concentration (c_ξ) as the logarithm of LD_{50}/g , namely as its reciprocal ($1/\log LD_{50}/g$), a very good agreement with the many available experimental data is obtained. The results reported by different research groups [14-19], in fact, are here presented and plotted according to the here evaluated parameters. While Fig. 1 is included in the text, others (Figs 2-6) are available at the website: www.iss.it/annali/ within the full text of this paper. Figs 1-5 refer to data obtained by inoculating the pathogenic prions in hamster 263K brains; they are shown in the

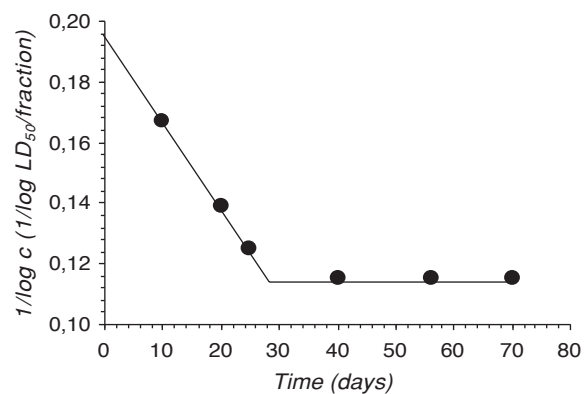


Fig. 1. - Variation of the intracerebral concentration of scrapie pathogenic prions Pr^{PSC} ($1/\log LD_{50}/\text{brain tissue fraction}$) as a function of time (days) following inoculation of Pr^{PSC} in hamster 263K brain (data from [14]).

Table 1. - Calculated values of α and β for the six sets of experimental data shown in Figs 1-6

Infecting agent	Animal model	Inoculated dose	Ref.	α	β
<i>PrP^{Sc}</i>	Hamster 263K	6.3 ($\log LD_{50}/\text{brain tissue fraction}$)	[14]	0.188	0.117
<i>PrP^{Sc}</i>	Hamster 263K	7.0 ($\log LD_{50}/\text{brain}$)	[15]	0.213	0.117
<i>PrP^{Sc}</i>	Hamster 263K	7.6 ($\log LD_{50}/g \text{ brain tissue}$)	[17]	0.165	0.125
<i>PrP^{Sc}</i>	Hamster 263K	7.6 ($\log LD_{50}/g \text{ brain tissue}$)	[16]	0.183	0.114
<i>PrP^{Sc}</i>	Hamster 263K	3.9 ($\log LD_{50}/0.05 g \text{ brain tissue}$)	[18]	0.487	0.171
<i>PrP^{CJD}</i>	Mice	5.5 ($\log LD_{50}/g \text{ brain tissue}$)	[19]	0.243	0.148

decreasing order of the dosage of inoculated *PrP^{Sc}* [14–18]. Fig. 6 (available at the cited website) refers to an additional set of data [19], also considered in the present study even if: a) the inoculated prion is not the scrapie prions, but the Creutzfeldt Jakob disease agent (*PrP^{CJD}*), and b) the inoculation was performed on mice and not on hamsters 263K.

It is self evident that all the plots show a similar pattern, constituted by two sharply different kinetic trends. The first part of the plots, where the experimental data show a straight line behaviour with a negative slope (exponential trend), confirms the Eigen theoretical approach of an autocatalytic replication of the pathogenic prions [11]: this means that the rate of replication is higher at lower initial concentrations (lower time values) of pathogenic prions; while the second part of the plots is strictly parallel to abscissa (Fig. 1) and roughly parallel to abscissa in the other cases, i.e. the behaviour becomes independent by the time. These evidences are, therefore, in agreement with a relationship that correlates the LD_{50} ($1/\log LD_{50}/g$) with the time (*days*). The first part of the plots is respective to the interaction between pathogenic and normal prions according to reaction (1), while the second part of the plots shows a trend almost invariant along time, being respective to the growth of the infective process in the brain tissue.

By extrapolating to zero time the trend of the values of the two different parts of the plot, respective hybrid kinetic constants, α and β , can be evaluated (see table).

As previously mentioned, the too different experimental conditions of the infective process, respective to data from [18], do not allow a homogeneous comparison with the other experiments. These latter conditions, in fact, refer to inoculations of concentrations of *PrP^{Sc}* three orders of magnitude lower than any other set of data, so that the progress of the infection is still detectable also 80 days after the inoculation. Data from [19] are also not homogeneous with the previous ones since they specifically refer, as already mentioned, to both a different pathogenic prion and to a different animal model [8, 19, 20]; so that the differences in α and β values could be explained merely in terms of a different experimental model.

It must be noted that when the experimental data (Fig. 1) refer to a more homogeneous material, i.e. they refer to selected fractions of brain tissue obtained after ultracentrifugation processes [14], the agreement with the model here proposed is even more convincing.

All data seem, however, to support the hypothesis of a change in the rate of advancement of the infection, showing a maximum value that marks a sort of “saturation” of the infective process that becomes independent of the number of pathogenic prions present in a given mass of brain tissue (i.e. the reaction rate becomes constant).

On the basis of such evidences it seems to exist a correlation between the velocity of the autocatalytic prions replication process and the degree of the advancement of the infection and, in all the cases after a period of about 30-50 days, a sort of “saturation” occurs in the tissue in which the pathogenic prions are concentrated [20]. This is, with a good approximation the incubation time lagging between the intracerebral inoculation of pathological prions and the first appearance of clinical symptoms.

The above mentioned effects, even if quite different from a practical point of view, seem to resemble the well known behaviour observed in many enzymatic reactions in which specific sites are involved, when the rate of the reaction changes from a first, or a second order, towards a zero order (maximum value of the reaction rate after the saturation of all active sites of the enzyme operated by the substrate).

In conclusion, although additional evaluation with supplementary experimental data is necessary to further confirm the validity of the approach here presented, we believe that it can be of some utility, not only as a simplified alternative to more complex models [21] but also for a preliminary, semi-quantitative evaluation of the efficacy of biomolecular strategies aimed to reduce the rate of advancement of the infection.

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