Volume 294, number 3, 155–157 FEBS 10452 December 1991

Hypothesis

Are prions misfolded molecular chaperones?

Jean-Pierre Liautard

INSERM U-65, Laboratoire de Biologie Cellulaire, Université de Montpellier II, C.P. 100; 34095 Montpellier Cedex 5, France

Received 27 August 1991; revised version received 10 October 1991

A theory has been developed that could explain prion infection. Prions could be molecular chaperones that are required for their own assembly. The theory has been deduced from an analysis of protein folding and consequences explored by computer simulations. Thermo-kinetic analysis of protein folding shows that a misfolded chaperone gives rise to new misfolded chaperones. Consequently such a protein could behave as a new kind of informative molecule and replicate misfolding according to a process similar to infection. A quantitative model has been derived from this hypothesis that displays the characteristics of prion infections. This hypothesis satisfactorily explains the three manifestations – infection, familial and sporadic – that are the characteristic features of all prion diseases.

Protein folding; Prion; Thermo-kinetics

1. INTRODUCTION

There is now considerable evidence that the infectious agents causing bovine spongiform encephalitis, scrapie, the human Gerstmann-Straussler and Creutzfeld-Jakob syndromes and other degenerative encephalopathies are pure proteins called prions [1]. However many biologists are unhappy with this idea, as it does not fit readily with the central dogma of molecular biology. The prion hypothesis is criticized on two counts: some workers have found nucleic acid in prion particles [2,3] and others [4] suggest that prions are not the causative agents of infection. However, many studies [5–7] have provided experimental evidence for the role of prions in the diseases. If prions really are the causative agents, their action should be explainable in terms of current biological concepts, rather than requiring a change in fundamental thinking. I have accepted two basic assumptions, (i) that prions are particularly stable proteins that cause illness by an infection-like process (see [1]), and (ii) that proteins similar or identical to prions are encoded by mammalian genomes [1]. Starting with an analysis of protein folding mediated by a molecular chaperone, a theoretical model is proposed that explains the major features of prion diseases.

Correspondence address: J.-P. Liautard, INSERM U-65, Laboratoire de Biologie Cellulaire, Université de Montpellier II, C.P. 100; 34095 Montpellier Cedex 5, France. Fax: (33) 67 14 33 38.

2. THEORETICAL ANALYSIS

Molecular chaperones are involved in directing the folding of other proteins. The best known are the heat-shock proteins. It has recently been shown that molecular chaperones can direct their own assembly [8,9]. The question follows naturally, what happens if a molecular chaperone is accidentally misfolded?

Correct folding is generally believed to provide the most stable protein (see [14]), however there is no proof for this hypothesis [12]. On the contrary, the conformation of folded prosubtilisin is unchanged by removal of a 77-residue N-terminal segment, but if this segment is removed before folding a new conformation is adopted [15]. Thus mature subtilisin has only kinetic stability. Metastable conformations do not violate kinetic theories of protein folding [13,16], and the very existence of molecular chaperones is a strong argument for such metastable conformation. Proteins requiring a molecular chaperone may remain in a metastable conformation for an infinite time if they do not meet their chaperone. This implies that the kinetics of folding is blocked by an energy barrier which is removed by the molecular chaperone, much like enzyme catalysis. These observations legitimate the use of kinetic theories of protein folding to analyze the mechanisms involved in protein folding promoted by molecular chaperones.

Analysis can be performed using the thermo-kinetic model of protein folding [13]. The thermodynamics of irreversible processes indicates that the speed of folding determines the final interactions between the amino acids [13]. Generalization to the folding catalyzed by molecular chaperones is straightforward. Consider the

case of an interaction between two molecules (an unfolded molecule I, and a folded molecule J, the molecular chaperone) of the same protein. The speed of interaction between two amino acids, one (a_i) from molecule I and the other (a_j) from the molecule J is governed by the relationship [13]:

$$V_{a_i \to a_j} = \sum_n \left[\left(\prod_m P^{ss}_{a_i+m} \cdot P^{ss}_{a_j+m} \right) (\Delta G_{a_i+n \to a_j+n}) \right] \quad (1)$$

where P^{ss} is the probability (according to Boltzman's law) of finding the amino acids in a conformation allowing interaction, n is the number of amino acids around a_i that influence the speed of association, and m is $E(1,n)$. As molecule J, the molecular chaperone, is folded, $P^{ss}_{a_j+m} = 1$ for all m, but as molecule I cannot fold without a molecular chaperone, its $P^{ss}_{a_i+m}$ is very low for all m. Hence, spontaneous correct folding does not occur, and the folding speed is governed by the molecule J. Thus the conformation of a_i and its neighbors should be that of the corresponding residues a_j and its neighbors [13]. Hence, if molecule J is misfolded, then molecule I will also be misfolded, and the new misfolded molecule should have the same structure as its molecular chaperone. The (misfolded) structure of the chaperone is transmitted to the new synthesized molecules.

This result implies that, under certain specific circumstances, a kind of biological information can be transmitted directly by proteins without the intervention of nucleic acid. Hence, the structure of a protein is informative by itself. Such proteins could be regarded as infectious organisms, replicating within a cells using, as do viruses, the cells' own components. Misfolding can be considered as a 'structural mutation' that propagates in the cell.

Are there evidences that prions are molecular chaperones?

First, molecular chaperones, as proteins, can be denatured, and prions lose their infectivity after denaturation [10], implying that their tertiary structure is of importance. Second, if prions are misfolded molecular chaperones, then they should exist in two differently folded forms. Lopez et al. [17] showed that prions synthesized in vitro could adopt two topological forms as a consequence of alternative folding. Thirdly, prion molecules present a zone containing glycine repeats [1,18]. Glycine is a very mobile amino acid, with all its conformations having about the same energy [12]. Hence these sequences have a low propensity to adopt spontaneously a definite secondary structure. Analysis according to the Gibrat et al. [19] method reveals that a third of the protein is poorly structured. These observations suggest a necessity of interaction with a folding partner to promote a define conformation.

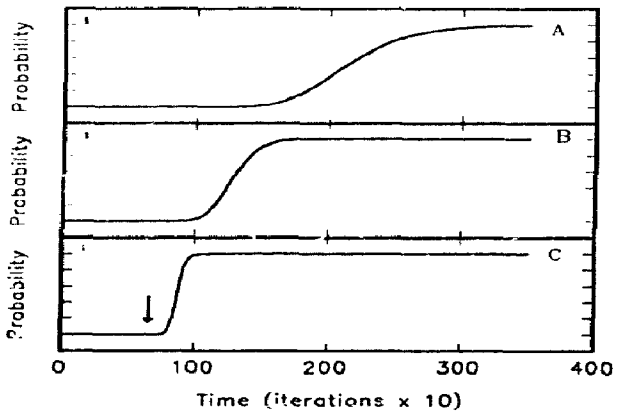

Fig. 1. Simulation of prion diseases appearance under the three specific cases: (A) sporadic, (B) genetic and (C) infectious. Invasion by prions was simulated by analysing the behavior of each newly synthesized molecule with a computer (see text). Time is proportional to the number of iterations. In B, the value of P_i is four-times the one used in figures A and C. In C, misfolded chaperones (prions) are added at time=600 iterations (arrow). For each case, simulation was repeated 5000 times and results were cumulated to give a kind of 'probability curve' that gives the probability to develop the prion disease.

3. A MODEL FOR THE SIMULATION OF PRION INVASION

According to the deductions presented above, a model can be developed to simulate prion invasion. Consider the number nP_t of prions (misfolded molecular chaperones) at time t when a new molecule is synthesized. This number will depend on the number of prions at time $t-1$ (nP_{t-1}). There is a probability (very low but not zero) of spontaneous misfolding (if folding is not performed on the chaperonine) termed P_i (it is easy to demonstrate that P_i is directly proportional to P^{ss} of eqn. (1)). Otherwise, the folding will be performed on a molecular chaperone and the probability of misfolding is $(nP_{t-1}/(n\mathrm{N}_{t-1} + nP_{t-1}))$, where $n\mathrm{N}_{t-1}$ is the number of correctly folded molecules. The elimination of the prion, as a physiologic degradation of protein, could also be calculated. Simulation was performed with computer by iteration. Numerous simulations were performed in order to obtain a probability of appearance as a function of the number of iterations (Fig. 1). The number of iterations is proportional to time. Fig. 1A depicts the sporadic apparition of prions after a long lag period. Clearly, according to the model, the mutations observed in degenerative brain illnesses favor prion diseases by increasing P_i (see section 4 for a discussion of this point). The calculations correctly simulate this phenomenon (Fig. 1B). Increasing four-times the probability P_i results in reduction of the invasion lag period of about 0.33. Infection is equivalent to a direct increase in nP. The results of the simulation presented in Fig. 1C

model such an infection. After injection of nP (arrow) the invasion appears very rapidly, without a lag period.

Clearly, specificity of prion diseases (infectious, sporadic, familial) are well simulated by the model.

4. ANALYSIS OF PRION DISEASES

Westaway et al. [1] suggests that prion diseases may fall into three categories: infectious, familial and sporadic. The above hypothesis provides a rational unified explanation for all three.

4.1. *Infectious disease*

In order to explain the infection, one necessary condition is that prions are stable enough to be taken into the organism, essentially undamaged, to become a chaperone. The stability of prions favors this hypothesis [1]. All the experimental evidence for the infectious properties of prions obtained to date has been from direct injection of protein preparation [1,6]. They will be used as molecular chaperones for the folding of newly-synthesized prion proteins. The misfolding of the prion results in the propagation of the error, producing increasing numbers of misfolded proteins which, because of their singular stability, will not be degraded by intracellular proteases. However, in regenerating tissues, misfolded protein will be diluted out from dividing cells and eliminated by cell death. Consequently, misfolded molecular chaperones will occur in large quantity only in non-dividing cells. This observation explains the brain localization of prion diseases. Furthermore, if the prion molecule is related to a molecule that is important for the physiology of the cell, there must be a feedback regulatory mechanism. A decrease in the number of correctly-folded chaperone molecules may well lead to an increased synthesis, giving rise to still more misfolded molecules. The converse is also true, i.e. injection of correctly folded molecular chaperone should partially reverse the effect of the prion. This opens the way to treating these diseases.

4.2. *Sporadic apparition*

This concept of prion diseases has other implications. A misfolded protein that arises accidentally could initiate cell invasion. Such accidental misfoldings are more likely with age, leading to increased probability of disease with age in a hitherto healthy person (see simulation Fig. 1A), as in human brain degenerative diseases.

4.3. *Explanation of the genetic transmission of the disease*

The accidental misfolding could be due to a mutation increasing the probability of spontaneous misfolding and giving rise to inherited diseases where illness appears more frequently in younger people (see Fig. 1B). Similarly, the recent onset of BSE could be due to genetic selection as well as direct infection.

Inbred strains of mice in which scrapie infections have short (NZB) and long (I/Ln) time-courses have a distinct prion gene allele [1,18]. The amino acid at codon 108 in the prion sequence is leucine in the NZB strain and phenylalanine in the I/Ln strain. This change would decrease the probability of alpha-helix formation, lowering $P^{ss}_{a_i}$ two-fold [19]. The highly conserved codon at position 189 is changed from threonine in the NZB mice to valine in the I/Ln strain, resulting in a three-fold increase in the probability of beta-sheet folding in a region which is otherwise likely to coil [19].

5. CONCLUSION

Structural information can be transmitted by protein. This concept offers a good frame to explain etiology of brain degenerative diseases caused by prions. Furthermore it suggests a treatment for these illnesses.

Acknowledgements: I thank M. Cellier for many helpful discussions on heat-shock proteins and E. Caron for general discussion and help with the manuscript.

REFERENCES

[1] Westaway, D., Carlson, G.A. and Prusiner, S.B. (1989) Trends NeuroSci. 2, 221–227.
[2] Kimberlin, R.H. (1982) Trends Biochem. Sci. 7, 392–394.
[3] Narang, H.K. (1990) J. Mol. Biol. 216, 469–473.
[4] Sharpe, A., Hunter, J., Chassler, P. and Jaenish, R. (1990) Nature 346, 181–183.
[5] Prusiner, S.B. (1982) Science 216, 136–144.
[6] Prusiner, S.B., Scott, M., Foster, D., Pan, K.M., Groth, D., Mirenda, C., Torchia, M., Yang, S.L., Serban, D., Carlson, G.A., Hoppe, P.C., Westaway, D. and DeArmond, S.J. (1990) Cell 63, 673–686.
[7] Safar, J., Wang, W., Padgett, M.P., Ceroni, M., Piccardo, P., Zopf, D., Gadjusek, D.C. and Gibbs, C.J. (1990) Proc. Natl. Acad. Sci. USA 87, 6373–6377.
[8] Cheng, M.Y., Hartl, F.U. and Horwich, A.L. (1990) Nature 348, 455–458.
[9] Lissin, N.M., Venyaminoy, S.Y. and Girshovich, A.S. (1990) Nature 348, 339–342.
[10] Prusiner, S.B., Groth, D.F., Bolton, D.C., Kent, S.B. and Hood, L.E. (1984) Cell 38, 127–134.
[11] McLachlan, A. (1976) J. Mol. Biol. 103, 271–298.
[12] Ghelis, C. and Yon, J. (1982) in: Protein Folding, Academic Press, New York.
[13] Liautard, J.P. (1990) C.R. Acad. Sci. 311, 385–389.
[14] Kim, P.S. and Baldwin, R.L. (1990) Annu. Rev. Biochem. 59, 631–660.
[15] Zhu, X., Ohta, Y., Jordan, F. and Inouye, M. (1989) Nature 339, 483–484.
[16] Brooks, C., Karplus, M. and Pettit, M. (1988) in: Proteins: A Theoretical Perspective of Dynamics, Structure, and Thermodynamics, p. 129, John Wiley and Son, New York.
[17] Lopez, C., Yost, C., Prusiner, S.B., Myers, R. and Lingappa, V. (1990) Science 248, 226–229.
[18] Carlson, G.A., Hsiao, K., Oesch, B., Westaway, D. and Prusiner, S.B. (1991) Trends Genet. 7, 61–65.
[19] Gibrat, J.F., Garnier, J. and Robson, B. (1987) J. Mol. Biol. 198, 425–443.