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Nature of cross-seeding barriers of amyloidogenesis

Dariusz Stępkowski✉ and Juliusz Bieniaś*

Laboratory of Molecular Basis of Cell Motility, Nencki Institute of Experimental Biology, Warsaw, Poland

The epidemics of bovine spongiform encephalopathy (BSE) several decades ago and present epidemics of chronic wasting disease (CWD) among cervids posed a threat of cross-species infections to humans or other animals. Therefore, the question as to the molecular nature of the species barriers to transmissibility of prion diseases is very important. We approached this problem theoretically, first developing a model of template-monomer interaction based on logical and topological grounds and on experimental data about cross-seeding of PrP 23-144 protein orthologs. Further, we propose that the strength of the cross-seeding barriers is proportional to dissimilarity of key amyloidogenic regions of the proteins. This dissimilarity can be measured by dissimilarity function we propose. Scaled on experimental data, this function predicts if cross-seeding can occur between different variants of PrP23-144. The resemblance of PrP23-144 cross-seeding barriers to the barriers of cross-species transmissibility of prion diseases is discussed. We suggest that a similar theoretical approach could be applied to predicting the occurrence of species barriers of prion diseases at least in part corresponding to the process of multiplication of infectious agent.

Key words: prion, amyloids, amyloidogenesis, cross-seeding, PrP23-144, cross-species barriers

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INTRODUCTION

Formation of amyloid fibrils *in vivo* occurs in more than 40 human diseases including: prion diseases (transmissible spongiform encephalopathies — TSEs), diabetes type 2, Alzheimer's disease, Huntington disease, Parkinson disease, and others (Chiti & Dobson, 2006). These maladies have very significant impact on the health of the human society. Therefore, the mechanism of amyloid fibrils formation, their structure and methods of avoiding undesired aggregation are very vigorously studied with special focus on the problem of cross-species transmissibility of prion diseases. To some extent this problem can be studied *in vitro* as occurrence of cross-seeding barriers of amyloidogenesis.

The architecture of amyloid fibrils is complex. The basic structural unit is formed from a part of a polypeptide chain in a β-strand conformation. These strands form a β-structure core and are located perpendicularly to the fiber axis (cross β-structure). Three topologies of the β-strands in the core are possible: parallel, antiparallel and mixed. The basic structural units form protofilaments which, on a higher level of structure organization, build fibrils. The structure of the protofilaments is stabilized by non-covalent inter and sometimes intramolecular interactions, whereas fibers are stabilized only by intermolecular interactions. The following stabilizing interactions are present in amyloid fibrils: hydrogen bonds which involve main chains and side chains, hydrophobic contacts including π-π stacking of aromatic side chains, and ionic interactions. The contribution of each of these types of interactions to the stabilization of amyloid fibrils has not been determined yet and probably depends on the type of amyloid fibril. However, it is thought that the hydrophobic effect, like in the process of folding of globular proteins, can be one of the main driving forces of aggregation to the amyloid state (Saiki *et al.,* 2005).

The present state of knowledge indicates that there is a very limited number of adjacent "key" aminoacid residues responsible for starting the conversion to the β-structure (Ciani *et al.,* 2002; Vanik *et al.,* 2004; Hall *et al.,* 2005; Esteraz-Chopo *et al.,* 2005; Kim & Hecht 2006; de Groot *et al.,* 2006; Kim & Hecht, 2008). The PrP23-144 prion protein is a very good example of this (Vanik *et al.,* 2004). This protein is a shortened human prion protein expressed as a result of a mutation of Tyr145 codon to a STOP codon. This mutation is linked to human disease of GSS phenotype (Gambetti *et al.,* 1999). It has been found by Kundu *et al.* (2003) that unlike the full length human prion protein, the recombinant human prion protein comprising residues 23-144 forms amyloid fibrils very easily. The amyloidogenic properties were mapped to residues 138-141 (Kundu *et al.,* 2003). The process of forming fibrils by this protein resembles self-seeded nucleation-polymerization reaction with a characteristic lag phase (Vanik *et al.,* 2004). Studying homologous recombinant proteins from mouse and hamster, Vanik *et al.,* (2004) found that residues 138 and 139 are responsible for barriers in cross-seeding between these proteins and human protein. Their results provide a basis for theoretical considerations about the mechanism by which PrP23-144 forms amyloid fibrils and about the molecular mechanism of seeding and its barriers. There appears a consensus in the literature that cross-seeding barriers may be related to divergence of aminoacid sequences of short sequence segments responsible for amyloidogenic properties of different proteins (Tartaglia *et al.,* 2005; Chen *et al.,* 2007; Bruce & Chernoff 2011). The data set of Vanik *et al.* (2004) however is the only experimental data set amenable for extensive analysis. First attempt to analyze these data was done by Tartaglia *et al.* (2005) who related cross-seeding barrier with predicted amyloidogenicity of amyloidogenic region of PrP23-144.

✉e-mail: d.stepkowski@nencki.gov.pl
*non-academic affiliation
Abbreviations: TSEs, transmissible spongiform encephalopathies

In the present study we propose a fibril seeding mechanism and a way of predicting the occurrence of cross-seeding barriers based on similarity comparison between the template and the monomer. Our theory is verified on the data about the cross-seeding barriers of PrP23-144 (Vanik *et al.,* 2004). A similar approach was used by Apostol *et al.,* (2011) who compared crystal structures of the amyloidogenic region of PrP23-144 from human, mouse and hamster and related the results to the occurrence of cross-seeding.

THEORY AND RESULTS

The template-monomer interaction on logical and topological grounds

Once a template of β-structure (in most cases part of a preformed fibril — an attachment face) is introduced to monomer solution, molecules of the monomer attach to it and convert to the a β-structure rich and become a next attachment surface of template (see Fig. 1 for explanation). In the case of a parallel in-register topology the new attachment surface is almost an exact copy of the template — attachment face, whereas in the case of other topologies it becomes a copy of the polypeptide chain fold. When the monomer has a aminoacid sequence identical to that of the protein forming the template, addition of a preformed template to monomer solution reduces significantly the duration of the lag phase. This process is called seeding of the monomer solution. Cross-seeding barriers may appear when the monomer does not exactly match the template amino acid sequence. This reasoning concerns the β-structured core of the fibril — its attachment face. Peripheral fragments have more conformational freedom and probably do not contribute significantly to the barrier. But they do contribute to the final morphology of the fiber (Jones *et al.,* 2011).

Topologies of key regions of template and monomer

We assume that, as the first molecular event, key amyloidogenic regions of the template and the monomer are involved in copying the fold of the template face layer conformation. There are four possible topologies of the arrangement of the polypeptide chains of the template and the monomer (see Fig. 2). The exact topology realized in solution will depend on the energy balance of interactions between the template and the monomer, both stabilizing and destabilizing the forming structure. Since the outcome of template-monomer interaction is copying of spatial arrangement of the template face an identical match of the sequence (realized in parallel and in-register topology) should be preferred unless it causes destabilizing interactions which would make it energetically unfavorable. A preference of parallel in-register topology is observed for amyloid fibrils of peptides larger than 20 aminoacids and is topologically justified (Margittai & Langen, 2008) as facilitating more hydrophobic contacts. We postulate that in case of amino acid sequence differences between the monomer and the template the probability of a occurrence of cross-seeding barrier will be proportional to the dissimilarity of the template and the monomer. Further, we propose to limit the similarity comparison to the key amyloidogenic region. Similar logics was applied by Apostol *et al.* (2011) to comparing crystallographic structures of amyloidogenic regions of PrP 23-144 from human, mouse and hamster and relating the results to the occurrence of cross-seeding barriers.

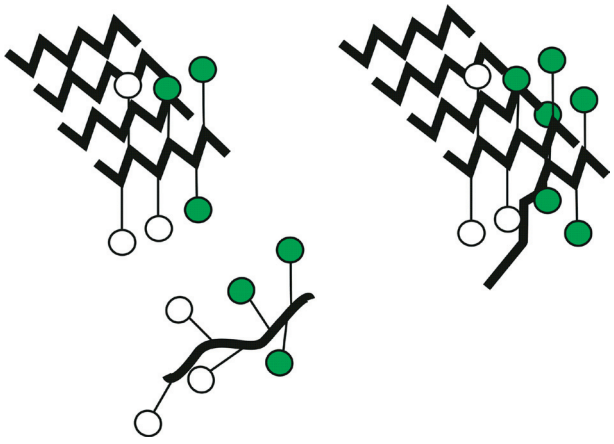

Figure 1. The idea of template-monomer interaction.
β-structured template forms interaction face to which monomer molecule attaches through key-residues side chains and then adapts its conformation to the template and forms new interaction face. Filled circles indicate side chains of key amino acids.

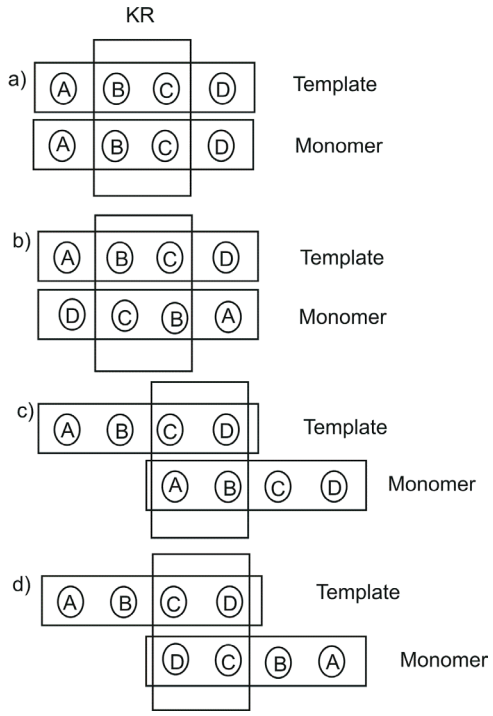

Figure 2. Four possible topologies of template and monomer polypeptide chains.
(a) parallel in-register; **(b)** antiparallel in-register; **(c)** shifted parallel; **(d)** shifted antiparallel. Rectangle indicates key amyloidogenic residues. Since the template-monomer interaction implies copying of the fold of the template polypeptide chain, two residues are a minimum for comparison. A, B, C, D consecutive positions in amino acid sequence.

Dissimilarity function

One of the aims of this study was to find a numerical way of measuring the ability of one protein to seed another protein. Intuitively, one expects that when a monomer has a more similar amino acid sequence to the template one, the cross-seeding will occur, while with a less similar sequence the cross-seeding will not occur The similarity is the highest when the monomer expected to form a

β-strand and its ortholog forming the template are in a parallel topology and in-register with each other.

When the monomer sequence is identical to the sequence of protein forming the template, seeding occurs. The lag phase is significantly shortened. When the sequences of the template and the monomer differ the question arises as to what the extent of similarity between sequences should be for cross-seeding to occur. Comparison of simple geometric objects like "segments" can be done by analysis of their length (a parameter describing their properties). Two "segments" with the same length are identical. Similarity can be measured by dissimilarity function S = |AB-CD|. When S = 0 "segments" are identical. When value of modulus is higher then "segments" are less similar. The problem of measuring the similarity of the template and the monomer is slightly different and can be approached by the following simplification: amino acid sequence can be approximated as a straight line with points representing each amino acid residue — or its side chains, to be more exact. Parameters representing properties of these side chains can be attributed to these points. Then, it is possible to compare the sequence of the monomer with the sequence of the template, by aligning them and measuring difference between parameters for each residue. Since the relation between the template and the monomer is by its nature asymmetric (the template is a reference point to which the monomer has to adjust its conformation), the dissimilarity function comparing the sequences of template and monomer should not be a modulus. It should rather indicate whether the template has a stronger β-structure forming potential than the monomer or *vice versa*. When the amino acid sequences of the template and the monomer are identical, the dissimilarity function should reach unique value indicating this fact. Such requirements are fulfilled by the following function which we call dissimilarity function:

$$S = \frac{\sum_{i=1}^{k} A_i \exp(T_i - M_i)}{k}$$

Where:
i — number of amino acid residue; A_i — weight of each residue contribution to amyloidogenic properties; T_i — parameter of i residue of template; M_i — parametr of i residue of monomer; k — total number of residues involved in template — monomer interaction (key residues); This function has the following properties: when $k = 1$; $A_1 = 1$ and $T_1 = M_1$ it reaches value equal to 1; when $T_1 > M_1$ it reaches values > 1; when $T_1 < M_1$ it reaches values < 1 and > 0; when $k = 2$, A_1 <0,1>; A_2 <1,0> the situation when $T_1 = M_1$ and $T_2 = M_2$ it will produce the value 0.5. Values of the dissimilarity function above 0.5 will indicate the template sequence of stronger β-structure forming potential of the cluster of key residues than does the monomer interacting with it. Values below 0.5 will indicate the opposite. A range of dissimilarity function values above and below 0.5 between certain border values will cover situations where cross-seeding is possible. Values above or below those border values will indicate non-occurrence of cross-seeding. Those crucial border values could be established from experimental data.

Testing dissimilarity function on experimental data

We assumed a parallel and in-register topology of β-strands in PrP 23-144 fibrils which recently was confirmed experimentally by Helmus *et al.,* (2011). It is known from the experiments that residues 138 and 139 are responsible for the amyloidogenic properties of PrP23-144 (Kundu *et al.,* 2003, Vanik *et al.,* 2004) Therefore, the dissimilarity function with k = 2 and sum of A_1 and A_2 equal to 1 was used by us to analyze experimental data concerning PrP 23-144 cross-seeding. We used two parameters of the side chains of residues 138 and 139: the hydrophobicity (according to Kyte & Doolittle scale, Kyte & Doolittle, 1982) and probability of forming β-structure (calculated by Garnier *et al.,* 1978,1996 method). We found that these two parameters correlate with the lag phase duration of fibril formation by human, mouse and hamster PrP23-144 (see Supplementary Material at www.actabp.pl). Figure 3A presents analysis of the dissimilarity function behavior with different weights A_1 and A_2 applied to the set of hydrophobicities of residues 138 and 139 of templates and monomers in the six described experimental settings of cross-seeding between human, mouse and hamster proteins (Vanik *et al.,* 2004). A comparison of the experimental data with the results of cal-

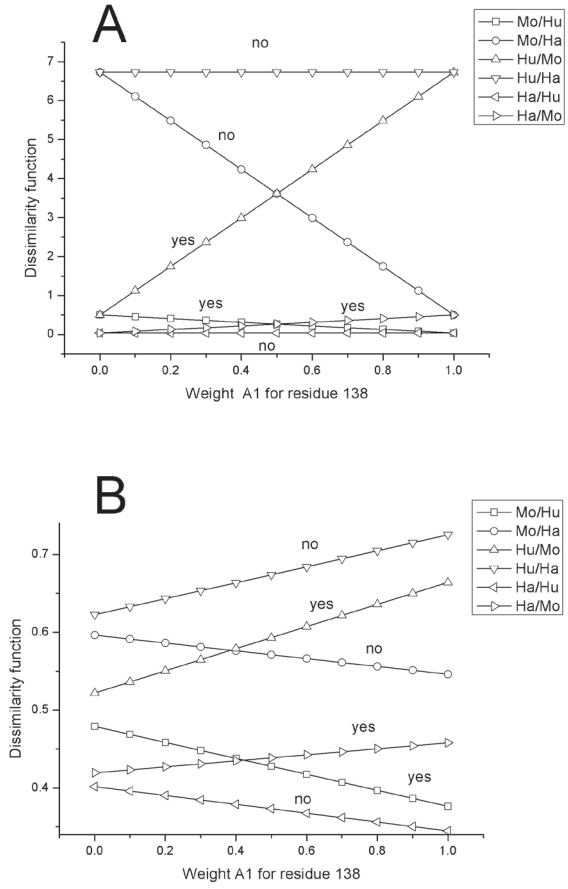

Figure 3. Determination of weights of parameters for residues 138 and 139 used in the dissimilarity function formula.
The value of similarity function was calculated for ten values of weights A1 (residue 138) and A2 (residue 139) in the range 0.1. **(A)** hydrophobicities compared, **(B)** probabilities of forming β-structure compared. Six experimental settings of Vanik *et al.* (2004) are presented and described in inset, seed being in front of the description. Hu — human; Mo — mouse; Ha — hamster. Lines marked"no" represent negative cross-seeding experiments. Reasonable range of values of similarity function correspond to the area "yes" marked between curves Mo/Ha and Ha/Hu and is restricted to common area of three positive seeding experimental settings.

culations leads to the conclusion that weight A_1 (for residue 138) should be between 0 and 0.5 with lower values producing wider range of S values indicating occurrence of cross-seeding. Figure 3B presents a similar analysis of the weights but with the use of the β-structure formation probability for residues 138 and 139. In this case the significance of residue 139 is even more pronounced than in the case of hydrophobicities since the range of A_1 allowing cross-seeding to occur is between 0 and 0.4. Further experimental data are necessary to allocate weights A_1 and A_2. more precisely. Among the curves from Fig. 3 Mo/Ha and Ha/Mo with the asymmetric outcome of cross-seeding are of special interest. The mouse protein is able to seed the hamster protein but not the other way round. For the Mo/Ha seeding variant, the dissimilarity function values are above 0.5 and for the Ha/Mo variant below 0.5 indicating in the second case the fact that the monomer has stronger potential of forming β-structure and hydrophobicity than the template. This coincides with the ability to cross-seed which can be explained in this way: once the template is formed (even from weak β-structure forming protein) the not-very-similar (in key amyloidogenic region) protein, but having intrinsic higher potential of forming fibrils is able to copy the fold of the template. In the former case, cross-seeding does not occur. It means that when the similarity is not high enough, even the stronger potential of β-structure formation of the template cannot cause a weak-β-structure forming monomer to multiplicate the fold of the template. The above reasoning illustrates the asymmetric nature of the template-monomer interaction and the dissimilarity function numerically indicates this fact.

DISSCUSION

Main conclusions and their experimental testability

The lag phase duration correlates negatively with the sum of hydrophobicities of residues 138 and 139 and also with the probability of forming a β-structure calculated for these residues (See Supplementary Material at www.actabp.pl). The higher the hydrophobicity or the β-structure forming potential the shorter is the lag phase. One may conclude that hydrophobic contacts between two or more PrP23-144 molecules through residues 138 and 139 are the starting event of formation of nuclei for amyloidogenesis. Because in the experimental setting other factors influencing lag phase duration like concentration and temperature were the same, the starting interactions are rate limiting. Growth of protofilaments and fibrils starts when nuclei (templates) are formed. It is justified to assume that a higher hydrophobicity of residues 138 and 139 means more likely hydrophobic interactions of these residues. When the preliminary interactions are more probable the formation of nuclei is easier and lag phase the shorter. Our reasoning is in line with recent experimental data concerning the influence of hydrophobicity of key residues on amyloidogenic properties of mutated β-amyloid 16-22 (Senguen *et al.,* 2011a; 2011b). A higher β-structure forming potential coincides with higher hydrophobicity (in the case of residues in question). Therefore, when the key-residues region forms β-structure more easily, the nuclei are formed faster. These conclusions can easily be tested in experiments with a mutated key-residues region of PrP23-144. The following scheme of PrP23-144 amyloid fibril formation can be proposed: two or more monomer molecules contact one another through key residues (in case of PrP23-144 residues 138-141).

These interactions facilitate formation of dimer or oligomer (minimal aggregate) which transforms part of its polypeptide chains into conformation of β-structure. The process starts in the key residue region and spreads most probably cooperatively along the chains further (Tsemekhman *et al.,* 2007; Hills & Brooks 2007). In the case of PrP23-144 it starts from the C-terminus and spreads toward the N-terminus. The template is formed.

Growth phase (template-monomer interaction)

Once the template is formed, monomers present in solution can interact with the template and acquire its conformation. The interaction occurs through key residues at first and monomers adapt to the template conformation becoming a new layer of the interacting face of the template.

First generation cross-seeding

A monomer with a key-residues sequence other than the sequence of the template may encounter a barrier of cross-seeding. The occurrence of the barrier can be deduced from simple calculations of similarity measure (dissimilarity function) scaled on a set of experimental data. In this paper we assumed parallel in-register topology of β-strands of the protofilament (Helmus *et al.,* 2011). However, a similarity comparison can probably be applied also to cases of antiparallel in-register and shifted parallel and antiparallel topologies assuming that key amyloidogenic regions of the template and the monomer are known.

Second generation cross-seeding

Surewicz and colleagues (Jones & Surewicz, 2005, Surewicz *et al.,* 2006) have put forward the idea of conformational adaptability to explain the phenomenon of second generation cross-seeding. In the light of our results conformational adaptability can be understood as the presence of a mediator protein which is similar to both the template and to the monomer non-cross-seeded by it. The similarity to the template makes it able to transform itself to a β-structure on the template and then as a new template — since it is also similar to the non-cross-seeded monomer — is able to transform it to β-structure. This corresponds well with the experimental results of Vanik *et al.* (2004) with mouse PrP23-144 unable in first generation seeding to cross-seed solution of hamster PrP23-144 monomers but when seeded with a hamster protein template becoming a new template able to seed hamster protein in second generation seeding. However, this example points to the fact that the second generation cross-seeding cannot be described by the parameters of the amino acid side chains only, it would probably require consideration of the spatial organization of the key amyloidogenic residues as used by Apostol *et al.* (2011) and numerical description of conformational adaptability.

Significance of described results for predicting barriers of prion diseases transmissibility between different species

The species barrier of prion diseases transmissibility is a complicated phenomenon. The biological basis of its occurrence is not fully understood. One may assume

that there are a few components to its presence during infection:
— influence of route of infection;
— influence of biological environment different in different species (the influence of biological environment on amyloidogenesis of non-prion proteins is reviewed by Bellotti & Chiti, 2008);
— molecular mechanism of infectious agent multiplication;
— molecular mechanism of neurodegeneration caused by infectious agent.

Assuming the validity of the "protein only" hypothesis of Prusiner (1982) it has been concluded that experiments of cross-seeding *in vitro* may resemble the first events of multiplication of an infectious agent of prion diseases in cross-species infections (Vanik *et al.,* 2004; Jones & Surewicz 2005; Surewicz *et al.,* 2006; Panza *et al.,* 2010) The question arises, whether the approach used in this paper to predict the occurrence of a barrier could be applicable to the case of full length prion proteins. However, it seems that the full length protein aggregates to fibrils through another mechanism than does PrP23-144. Recent results of Surewicz and colleagues (Lu *et al.*, 2007; Cobb *et al.,* 2008) about the structure of human PrP90-231 amyloid suggest that other key residues may be involved in amyloid formation than for PrP23-144. Therefore, our approach cannot be applied straightforwardly to this case. Predicting barriers for full length prion protein cross-seeding would require first to find the key-residues region of the prion protein molecule and to develop a numerical description of parameters facilitating the formation of the nucleus (template) and further growth of the protofibril. These parameters could be further used to calculate the values of dissimilarity function between key amyloidogenic regions of prion proteins from different species. The complete approach to this problem should also accommodate the existence of strains of prions which have the same or very similar amino acid sequence but multiplicate with different rates. This phenomenon may reflect another key region being involved in each strain and existence of sterical or structural impediments stemming from the different morphology of fibrils of different strains changing the kinetics of their multiplication. Different key amyloidogenic regions have recently been proposed as an explanation of the polymorphism of β-amyloid fibrils (Colletier *et al.*, 2011).

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