Structure of infectious prions: stabilization by domain swapping

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ABSTRACT A candidate structure for the minimal prion infectious unit is a recently discovered protein oligomer modeled as a β-helical prion trimer (BPT); BPTs can stack to form cross-β fibrils and may provide insight into protein aggregates of other amyloid diseases. However, the BPT lacks a clear intermonomer binding mechanism. Here we propose an alternative domain-swapped trimeric prion (DSTP) model and show with molecular dynamics (MD) that the DSTP has more favorable intermonomer hydrogen bonding and proline dihedral strain energy than the BPT. This new structural proposal may be tested by lysine and N terminus fluorescent resonance energy transfer (FRET) either directly on recombinant prion protein amyloid aggregates or on synthetic constructs that contain the proline/lysine-rich hinge region critical for domains to swap. In addition, the domain swapping may provide 1) intrinsic entanglement, which can contribute to the remarkable temperature stability of the infectious prion structure and help explain the absence of PrPSc monomers, 2) insight into why specific prolines are potentially relevant to three inherited forms of prion disease, and 3) a simple explanation of prion strains assuming the strain is encoded in the monomer number of the oligomers.—Yang, S., Levine, H., Onuchic, J. N., Cox, D. L. Structure of infectious prions: stabilization by domain swapping. *FASEB J.* 19, 1778–1782 (2005)

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MODEL β-HELICAL PRION TRIMER

The protein-only model for prion diseases remains the leading candidate to explain the infectious form of the disease (1), and this has been significantly strengthened by the successful production of disease from synthetic prion protein aggregates (2). Clearly, the understanding of prion disease pathology, propagation within the body, and therapeutic approaches will be greatly enhanced by a detailed knowledge of the structure of the minimal infectious protein unit.

The discovery of areal aggregates of infectious prion protein oligomers in purified brain extracts from mice has inspired a leading candidate model for the minimal infectious unit, a trimer in which large portions of the N terminus are converted to left-handed β-helices (3, 4). We call this the β-helical prion trimer (BPT) model. Figure 1A shows a top view of the BPT in the β-helical region. This BPT displays a 3-fold symmetry consistent with maps of the electron density difference between the PrP 27-30 oligomers and the “miniprion” PrPSc106 oligomers. The BPT can easily be stacked to form filaments, which can then be wound into amyloid fibrils. The model trimer also shares detailed structural features with the bacterial protein carbonic anhydrase trimer (5). Inspired in part by this model, others have explored the possibility that the β-helix plays a role in other amyloid structures such as aggregates of polyglutamine peptides (6), β-amyloid peptides (7), and yeast prion-like proteins (8).

However, it is not clear in the BPT model what holds the trimer together. The carbonic anhydrase trimer, which was the template for the BPT model is in fact held together by intermonomeric covalent bonding of zinc ions (5), which are not present in PrPSc. It was suggested that a hydrogen bonding network is responsible for this binding (4), as in the case of the bacterial β-helical trimer serine acyltransferase (SAT) (9), but no effort was made to test this idea quantitatively. Moreover, the yellow loops of the BPT model (which are highly conserved (10)) shown in Fig. 1A, B and expanded in Fig. 1C contain two prolines, and prolines tend to disfavor this type of bending.

HYPOTHESIS: A DOMAIN-SWAPPED PRION TRIMER IS THE MINIMAL INFECTIOUS UNIT

We hypothesize that a stable oligomeric structure for the infectious prion is the domain-swapped trimeric prion (DSTP) of Fig. 1B, which can increase hydrogen bonding and reduce elastic energy relative to the BPT structure proposed earlier. Further, we conjecture that the DSTP structure may be relevant to 1) the unusual temperature stability of the prion protein and the lack of observation of PrPSc monomers (both due to the entanglement of the DSTP structure), 2) the kinetics of at least three inherited forms of human prion disease, and 3) the encoding of prion strains in conformation.

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Concern with stability of the BPT model led us to modify it by domain swapping (11–13), especially since prolines are present in the hinge or bridging regions of several proteins that domain swap (14). For example, in p13suc1, the strain on two prolines in the hinge modulates the equilibrium between monomers and domain-swapped dimers (15, 16).

We constructed our DSTP model of Fig. 1B from the proposed BPT structure (we thank C. Govaerts for providing his coordinate file) by reorienting the monomers to place the loop regions near the trimer center providing his coordinate file) by reorienting the mono-

mers to place the loop regions near the trimer center and swapping residues 89-99 to a neighboring -helix in a cyclical fashion, with the BPT loops serving as hinges. This DSTP model straightens these loops, adds more possible hydrogen bonds in the loop region, and preserves the putative monomer-monomer spacing and 3-fold symmetry of the electron density maps (3). We ran all-atom, explicit solvent MD (see Appendix for details) on energy minimized DSTP and BPT structures using AMBER8 (http://amber.scripps.edu). As a control, we applied the same protocol to the serine acyltransferase (SAT) -helical trimer, which has no strained prolines and for which there is direct crystallographic evidence for intermonomer hydrogen bond-mediated cohesion (9). We also carried out two other simulation tests: 1) we examined the stability of the DSTP model with the inclusion of the C terminus -helices proposed in ref 4, and 2) we carried out three distinct 1-ns runs for both the BPT and DSTP structures to make sure there is no special circumstance with regard to individual simulations.

We have concluded that the DSTP is significantly more stable than the BPT and therefore a more promising candidate structure. First, we have enumerated direct and water-bridged hydrogen bonds between monomers in the BPT model, our DSTP model, and the SAT structure (see Appendix), and carried this out for three separate MD runs on each structure. We show the direct hydrogen bond counts for three different 1-ns runs of the BPT and DSTP structures in Fig. 2. For the BPT, we find 2 ± 1, 5 ± 1, and 2 ± 1 direct hydrogen bonds for the three runs (averages over the last 100-ps of simulation time), and at the last simulation time 0, 0, and 1 water-mediated hydrogen bonds. For the DSTP, we find for all three runs 9 ± 1 direct hydrogen bonds for the last 100-ps of simulation time and 2, 1, and 1 water-mediated hydrogen bonds in the last simulation snapshot. In the one BPT case with a relatively high number of direct hydrogen bonds, the N terminus from one monomer has contacted with the N terminus region of another. In general, the BPT hydrogen bonds form around Q89 only, while the DSTP hydrogen bonds form in the hinge region. Note that Fig. 2 shows direct hydrogen bonds formed in the monomer-monomer interface for the DSTP to generically increase with time while those from the BPT generically decrease with MD time. In contrast to the BPT, the simulated -helical portion (F140-A237) of the SAT trimer has 11 direct and 3 water-bridged hydrogen bonds (after two stage equilibration with no restraints).

Second, we remark that the domain swapping also relaxes the P101 and P104 dihedral energy by ~1.0 kcal/mol after 1-ns of MD. Hence, the domain swapping helps relieve elastic strain as anticipated.

Third, we have verified that the DSTP structure for the PrP\textsuperscript{Sc}106 can accommodate the C terminus -helices without affecting stability. This is a nontrivial point, since a rigid rotation of the monomers within the BPT is not possible due to steric constraints. As shown in Fig. 3, we find that by “peeling off” one triangular edge (residues 133-140) of the lower (C terminus) end of each -helix to form a random coil segment that we can link to the -helices that begin at residue 177, with no resultant change in direct hydrogen bonding structure.

Finally, high temperature MD provides direct evidence for BPT instability; at 500K, the DSTP remains

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**Figure 1.** A) Proposed -helical structure of the truncated PrP\textsuperscript{Sc}106 prion trimer (BPT) taken from ref 4 (residues G89-F140). B) Structural model of domain-swapped trimeric prion (DSTP) of the present paper. C) The proposed hinge region expanded from the yellow loops in panels A, B.

**Figure 2.** MD simulations show that the nonswapped BPT (A) has less direct hydrogen bonds in the interface region than the domain-swapped DSTP (B). In both cases we have simulated residues G89-F140. Different colors correspond to three different 1-ns MD runs in each case initialized at different initial temperatures (see Appendix).
compact and entangled, while the BPT monomer-monomer separation nearly doubles. Principal component analysis, which identifies the amplitude and character of the dominant fluctuations, shows similar BPT unbinding tendencies at 300K as in the 500K simulation, but no unbinding tendencies for the DSTP. Figure 4 shows the radius of gyration in the 500K runs as a function of simulation time. Within the 1-ns window of simulation, the BPT radius appears to keep growing, reaching about twice that of the apparently more stable DSTP structure. The α-helices have no effect on the stabilization induced by the domain swapping.

POSSIBLE RELEVANCE TO INHERITED DISEASES AND PRION STRAINS

Finally, we discuss the possible relevance of the DSTP model to inherited diseases and strains. First, proline point mutations yield heritable Creutzfeldt-Jakob disease (P105T for humans, P104T for mice) or Gerstmann-Sträussler-Scheinker disease (P102L, P105L for humans, P101L, P104L for mice; see chapter 14, ref 1). Transgenic mice with P101I have even greater disease susceptibility whereas those with P101Y have somewhat less susceptibility than P101L (K. Nazor and G. Telling, private communication). We note that the centrality of these sites in the DSTP hinges affords possible burial of the relatively hydrophobic I, L, T, and Y side chains and a potential speed up of trimer formation through increased hinge flexibility. Moreover, the insertion of relatively hydrophobic residues into the unstructured region of the normal PrPC protein will raise the energy of this structure. Indeed, it is already known that the P102L mutation is marginally less stable than the wild-type protein (see p. 694, ref 1). A decrease in the stability of the cellular protein form relative to wild-type monomers coupled to an increase in stability of the scrapies form (associated with more effective hydrophobic burial of the mutant residue) should lead to more rapid conversion kinetics via ramping of the overall free energy surface slope in favor of the scrapies form.

In Fig. 5 we illustrate two ways in which strains might breed true if the monomer content within an oligomer encodes the strain information. With this assumption, strains can breed true either by 1) oligomer conversion and crystallization on membranes (Fig. 5A), with local shape matching favoring homogeneous growth in a manner analogous to the oriented aggregation of nanoparticles (17), or 2) via interneuronal, epitaxially templated conversion across the synaptic cleft or interneuronal gaps (Fig. 5B). The plausibility of the latter conjecture rests on the length of unstructured N terminus PrPC, which is ~100 residues, or ~30 nm stretched out, easily sufficient to span the interneuronal gaps of 10–20 nm. Both of these models would enjoy enhanced conversion kinetics in the presence of unconverted or “soft” oligomers of PrPC (18); the interneuronal mechanism in this case would allow for exponential growth via oligomeric autocatalytic conversion. We note that a different domain swapping model

Figure 3. A) Model for DSTP PrPSc106 (with residues G141-H176 deleted) and α-helices included. Residues 133-140 have been removed from the lower rung of the β-helices for each monomer, converted to random coil, and attached to residue 177 of the α-helices. B) Number of direct hydrogen bonds for the DSTP structure with intact α-helices. The α-helices have no effect on the stabilization induced by the domain swapping.

Figure 4. The entanglement due to domain swapping introduces additional stability for the DSTP relative to the BPT as revealed by high temperature (T=500K) simulations, where the radius of gyration of the BPT increases more rapidly than that of the DSTP, which reflects unbinding of the BPT. Principal component analysis at 300K reveals a similar unbinding tendency.
for strains has been conjectured previously (19); sepa-

rately, domain-swapped prion dimers have been crystal-
lized (20). The latter result is consistent with prion
oligomerization via intermolecular disulfide bonding as
has been shown by redox induced prion fibrillization in
vitro (21), although only intramolecular disulfide
bonding has so far been observed in vivo (22). Other
specific domain swapping mechanisms than those we
have discussed here may play a critical role in amyloid
fibril formation for prions as has been observed for
other proteins (24–26). For the PrP 27-30 scrapies
form, the large loop (residues E145-R163) of ref 4)
made from some of the deleted residues of the
PrPSc106 structure could serve as a hinge region for
alternative domain-swapped structures.

PROPOSED EXPERIMENTAL TESTS

While the DSTP structure may ultimately be testable
directly on recombinant infectious prion oligomers, as
have been synthesized by Prusiner and collaborators
recently (2), we first propose testing an analog system
by FRET labeling. Poly-Q peptides are of considerable
interest because of the polyglutamine diseases (espe-
cially Huntington’s). The “critical” length near 36
glutamine repeats for disease is suggestive of a left-
handed β-helix structure, as discussed elsewhere (4, 6,
27). The key experiment we propose is to construct
synthetic peptides of the form Q12(KPSKPK)Qm,
where m = 24–36. The goal is to label the N terminus
Q’s in half the peptides with a donor, the K’s with a
different donor, and in the other half put in N termin-
us Q acceptors and K acceptors. If the argument about
stress relaxation and hydrogen bond stabilization works
for the prion, it should also work here. There should be
intense K-K FRET given intermonomer separations of
the side chains by ~4 Å if the domain swapping arises.
There will also be weak N terminus-Q- N terminus-Q
FRET. Presumably there could be an N terminus-Q-to-
N terminus-Q FRET signal arising with time if the
trimers are first formed and then they fibrillize, since
the corresponding distances would get reduced by
~60% case assuming stacking of the β-helices. If this
works on the synthetic peptide it should be attempted
on recombinant prion protein prior to in vitro aggre-
gation as per ref 2. We would anticipate little interfer-
ing contributions from other lysines in the prion pro-
tein sequence that are far from the hinge region.

SUMMARY

We propose a domain-swapped trimer model for the
minimal infectious prion oligomer, and note that the
domain swapping stabilizes the oligomer relative to the
earlier proposed model by reducing stress in proline
containing loops, increasing intermonomer hydrogen
bonding, and promoting entanglement. We conjecture
that domain swapping may play a role in explaining the
temperature stability of the scrapies form of the prion
protein, the absence of PrPSc monomers, inherited
prion disease phenotypes associated with mutating pro-
lines in hinges of the DSTP, and that conformation may
be encoded in the monomer number in a given oli-
gomer. Finally, we propose experimental tests based on
FRET labeling of principally lysine residues near the
crucial pralines, which should produce intense FRET
response if the domain swapping is present.

Appendix

The simulations were carried out with the AMBER8 molecu-
lar dynamics package (28) with the AMBER parm99 force
field and explicit TIP3P waters, following these procedures.
1) Before we start a molecular dynamics simulation, we perform
an energy minimization of 2000 steps to partially relax the
entire molecular system. 2) We perform a two-stage equilibra-
tion to further relax the protein and the surrounding solvent.
In the first stage, we start the system from a low temperature
of approximately 100K and gradually heat up to 300K over 20-ps of simulation time. We perform this stage of equilibration with the volume held constant. In the second stage, we equilibrate the system using pressure and temperature control to adjust the density of water to experimental values. We have equilibrated the system for a total of 40-ps. During the first two steps, the backbone heavy atoms of the β-helical (and also the α-helical if applicable) portions are positionally restrained using a harmonic potential. The hinge loops and the regions linking the β-helical and α-helical domains are allowed to freely move. To achieve the three different MD runs for the BPT and DSTP structures, we start with different initial temperatures that seed a different run through a different initial thermal distribution of atom velocities. Finally, we carry out a 1-ns production run at constant pressure and temperature with no positional restraints. For the high temperature (T = 500K) simulations, the production runs are performed at constant volume with a reduced time step of integration to prevent any possible system blowup.

Hydrogen bonds are calculated based on the following criteria: 1) direct hydrogen bonds in interface are counted with a donor-acceptor distance of 3.5 Å and a hydrogen-donor-acceptor angle of 60°; and 2) water-bridged hydrogen bonding in interface is counted if a water molecule couples by hydrogen bonding in interface is counted if a water molecule couples by donor-acceptor angle of 60°, and donor-acceptor distance of 3.5 Å and a hydrogen-donor-acceptor distance of 3.5 Å and a proton. In three-dimensional domain swapping: a mechanism for oligomer assembly. Structure 5, 391–401

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