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Volume and energy folding landscape of prion protein revealed by pressure

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Abstract

The main hypothesis for prion diseases proposes that the cellular protein (PrP^C) can be altered into a misfolded, ß-sheet-rich isoform, the PrP^{Sc} (from scrapie). The formation of this abnormal isoform then triggers the transmissible spongiform encephalopathies. Here, we discuss the use of high pressure as a tool to investigate this structural transition and to populate possible intermediates in the folding/unfolding pathway of the prion protein. The latest findings on the application of high pressure to the cellular prion protein and to the scrapie PrP forms will be summarized in this review, which focuses on the energetic and volumetric properties of prion folding and conversion.

Key words
- Prion
- High pressure
- Structural conversion
- Aggregation

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Introduction

Since its discovery by Stanley Prusiner in 1982, the prion protein (PrP) has received much attention in the field of medical science (1). This protein is implicated in the neurodegenerative diseases called transmissible spongiform encephalopathies and thus far is believed to be the major agent (or only agent) that causes these affections (2). The onset of a transmissible spongiform encephalopathy is triggered by the conversion of an α-helical isoform, which is the cellular PrP, denoted PrP^C, into a ß-sheet-rich form, the prion scrapie, denoted PrP^{Sc} or PrP-res (from protease-resistant) (2-5). The PrP^C is found anchored to the cell membrane mainly in cells of the central nervous system by a glycosylphosphatidylinositol bridge and is rich in α-helical structure and highly soluble (6). In contrast, PrP^{Sc} is mostly insoluble, presents partial resistance to proteolysis and has a greater ß-sheet content than PrP^C (6,7). Although PrP^{Sc} may exist in variable truncated forms *in vivo*, due to its partial resistance to proteolysis (8), both forms are generally derived from the same primary sequence of the prion protein.

The mechanism of conversion from PrP^C to PrP^{Sc} is still under study, and although most research groups suggest that only the presence of the scrapie form is necessary to induce PrP^C to acquire the misfolded conformation, some researchers have shown that other macromolecular candidate accessory factors, such as heparan sulfate (9-12), proteins (13,14) and nucleic acids (15-19), may be involved in this conversion.

In the last two decades, several groups have published studies on the thermodynamic and structural properties of this unusual protein (20-27). These investigators have described common peculiar solution conditions that favor *in vitro* formation of

the polymerized, ß-rich and proteinase K-resistant PrP structure, which involve incubation of recombinant PrP under acidic conditions (pH 4 to 6) and at denaturing or subdenaturing concentrations of urea or guanidinium salt.

Another approach to the understanding of prion conversion is the use of physical variables - heat, cold and high pressure - to assess the energetic and volumetric values involved in conformation changes (28-32). Another goal of the knowledge of such variables was to reduce or even to abolish infectivity of brain samples containing prion scrapie using these combined treatments, by high temperature autoclaving (33,34).

An important relatively new tool for investigating the conformational transitions of PrP is high pressure, which has an advantage over other methods because its perturbation of macromolecules in solution depends solely on the volume change of the process under study (35). High pressure favors the formation of structures with smaller volumes, and the application of pressure generally hydrates the hydrophobic interior of proteins (35,36). Therefore, proteins with a large volume fraction of solvent-excluded cavities are highly sensitive to pressure (35,37,38), a fact that makes this variable unique for exploring hydration, packing and volumetric properties of proteins. Moreover, pressurization of a particular protein may allow one to populate intermediate species in the folding pathway and to study these conformations, which otherwise would be difficult to isolate by other approaches (39). Since the 1980's, high pressure has been used successfully to explore protein folding (40), assembly (41), dynamics (42), and structure (43). More recently, high pressure has also been applied to misfolded proteins that form aggregates and amyloids (32,38,44-46).

In this review we will focus on the use of high pressure to dissect in more detail the prion conversion from the α-helical to the ß-sheet-rich isoform and folding/unfolding of this protein, which presents one of the major challenges in the neuroscience, biochemical and biophysical fields.

Use of high pressure to abolish prion infectivity

Although high temperature autoclaving has been used in the last decade to try to reduce or abolish prion infectivity from infected brain-tissue (33,34), high pressure has only been applied for this purpose a few years ago. At first inspection, the application of pressure to infected material seems to be promising, but it only produces significant results when combined with very high temperature (47,48). When several pulses of pressures - pressure pulse technology - above ~700 MPa were applied to the 263K scrapie strain adapted to hamsters, a high reduction of infectivity levels was obtained, but always coupled with high temperature incubation of the material (47). A similar result was obtained recently with the same prion strain (48,49). Crude brain homogenates of terminally diseased hamsters infected with the 263K scrapie strain were treated at 60ºC with pressures above 500 MPa (48) or at 800 MPa (49), and the titers of prion infectivity were significantly reduced when treated samples were inoculated into healthy hamsters. Interestingly, when this group applied high pressure (800 MPa) at 60ºC to purified prion fibrils, resistance to proteinase K digestion and infectivity were retained, suggesting the existence of distinct ß-structures in the PrP, sensitive or highly resistant to pressure (49). Besides, even for the assays in which prion titers were significantly reduced, abolition of total prion infectivity could not be achieved in any reported study.

Use of high pressure to search for alternative prion conformations

Several studies have been performed on the thermodynamics and stability of the PrP,

describing interesting features of this protein (21,50-52). Thus, the main characteristics of the PrP behavior can be clearly determined, but normally the results are quite variable and it is important to carefully analyze the type of prion construction studied in conjunction with the unfolding treatment utilized (see summary of the data in Table 1).

Application of pressure to proteins can reveal different conformations during the unfolding transition, which cannot be obtained by other methods. Soluble native Syrian hamster PrP ($ShaPrP^{90-231}$) could be rescued by pressure (200 MPa and returning to atmospheric pressure, at 70ºC) from temperature-induced ß-sheet-rich aggregates (45). Interestingly, when pressure was raised over 400 MPa at 40ºC, a conformational interme-

Table 1. Overview of prion isoforms characterized by high pressure.

Pressure/ temperature	Prion construct	pH conditions	Initial and final structural properties	Thermodynamic properties	Ref.
3 to 200 MPa 30ºC to -20ºC	Native, soluble $SharPrP^{90-231}$	pH 5.2	Cold denaturation of ShaPrP achieved	ΔG^0 values were calculated for individual residues and ranged from 3.0 to 5.3 kcal/mol	29
1 to 350 MPa 25ºC	$mrPrP^{121-231}$	pH 7.0 4 M urea	No complete unfolding achieved; stabilization of a partially folded intermediate	ΔV = -39 ml mol^{-1} $^a\Delta G_{int}$ = 2.2 kcal mol^{-1}	59
350 MPa 25ºC to -9ºC	$mrPrP^{121-231}$	pH 7.0 4 M Urea	Cold denaturation achieved under pressure with 4 M urea	$^b\Delta G_U$ = 1.1 kcal mol^{-1}	59
50 to 275 MPa 70ºC	Temperature-induced aggregates of $SharPrP^{90-231}$	pH 7.0	Pressurization allowed recovery of native, non-aggregated ShaPrP	Not calculated	45
50 to 600 MPa 40ºC	Native, soluble $SharPrP^{90-231}$	pH 7.0	Pressure-induced unfolding obtained	ΔG_U = 3.93 kJ mol^{-1} ΔV_U = -31.9 ml mol^{-1}	45
1 to 1000 MPa 25ºC	Native, α-helical $mrPrP^{23-231}$	pH 7.5	Pressure-induced unfolding achieved	ΔG^0_U= 5.4 kcal mol^{-1} ΔV = -29.2 ml mol^{-1} $p_{1/2}$ = 540 MPa	30
1 to 1100 MPa 25ºC	Aggregated, ß-sheet-rich $mrPrP^{23-231}$	pH 7.5	Pressure-induced unfolding achieved	ΔG^0_U = 2.8 kcal mol^{-1} ΔV = -43.6 ml mol^{-1} $p_{1/2}$ = 280 MPa	30
1 to 600 MPa 40ºC	$SharPrP^{90-231}$	pH 8.5	rPrP aggregates above 450 MPa	Not calculated	32
Transient treatment at 600 MPa, 40ºC	$SharPrP^{90-231}$	pH 8.5	Formation of amorphous aggregates after pressure release	Not calculated	32
Overnight incubation at 600 MPa, 40ºC	$SharPrP^{90-231}$	pH 8.5	Formation of amyloid aggregates after pressure release	Not calculated	32

[a]Free energy change corresponding to the transition from the rPrP state at 4 M urea, atmospheric pressure to the partially folded pressure-denatured state. [b]Free energy change of the transition from the pressure-denatured intermediate state to the fully unfolded state. $p_{1/2}$ = pressure value corresponding to 50% of the transition; SharPrP = Syrian hamster recombinant prion protein; mrPrP = murine recombinant PrP.

diate was populated, revealing other advantages for the use of this approach.

Kuwata and co-workers (29) have taken advantage of high-pressure nuclear magnetic resonance to monitor in real time the effect of pressure on the three-dimensional recombinant ShaPrP$^{90-231}$ structure. They could only reach the rPrP denatured state by cooling the sample to -20ºC at 200 MPa, but at 30ºC and 200 MPa a locally disordered rPrP intermediate was revealed (29) and more information was published recently (31). The yeast PrP Ure2 also underwent cold denaturation induced by pressure (200 MPa) (28).

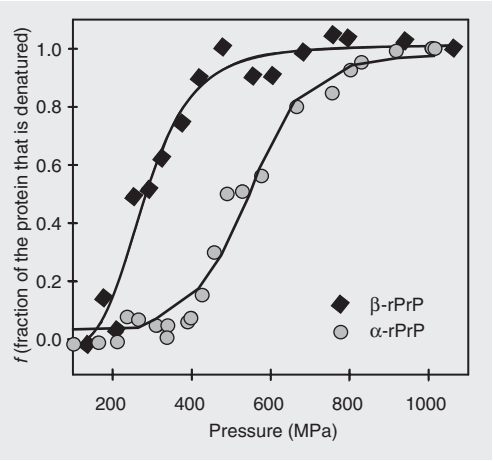

Figure 1. α-rPrP and ß-rPrP display different stabilities against pressure. The transition values as a function of pressure were obtained from the native α-rPrP α-helical secondary structure and from ß-sheet changes for ß-rPrP (obtained from Ref. 30). The extent of denaturation (f) was calculated as follows: $f = (IR_{obs} - IR_{initial}/IR_{final} - IR_{initial})$, where IR_{obs} is the observed IR intensity value at pressure p, $IR_{initial}$ is the IR value at 0.1 MPa, and IR_{final} is the final IR intensity value of the pressure-induced unfolding curve (from Ref. 30).

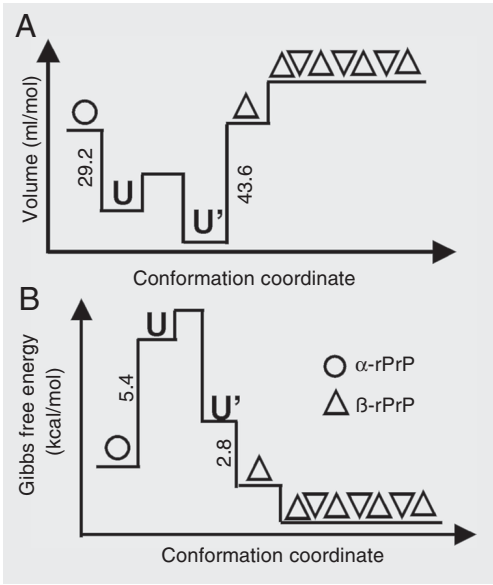

Figure 2. Volume (A) and Gibbs free-energy (B) diagrams of α-rPrP and ß-rPrP. α-rPrP (circle) and ß-rPrP (triangle) pressure-denatured states are denoted U and U', respectively. The numbers indicate volume (ml/mol) (A) and Gibbs free-energy of unfolding (kcal/mol) (B) determined for α-rPrP and ß-rPrP pressure-induced unfolding (from Ref. 30).

We have also used high pressure to try to reach the rPrP unfolded state. Recently, we confirmed that pressures above 500 MPa promoted denaturation of recombinant mouse PrP (rPrP$^{23-231}$) (30). The effects of high pressure on ß-sheet-rich rPrP aggregates (ß-rPrP), which were obtained by thermal treatment, were also investigated (30). The use of high-pressure Fourier-transform infrared spectroscopy (53-56) allowed us to probe the secondary structure of the protein during pressurization. We found that, whereas α-helical rPrP undergoes aggregation at high temperature into a ß-sheet-rich structure, it is markedly resistant to pressure, displaying almost no change in secondary structure up to 400 MPa, as reported previously (29). However, the ß-rPrP aggregates were highly susceptible to pressure and dissociated at pressures below 400 MPa (Figure 1). We showed for the first time denaturation of recombinant full-length PrP by high pressure without the use of temperature or denaturants and also reported that newly formed aggregates are less hydrated and have more cavities than native PrP and late aggregates. We calculated the thermodynamic parameters of the α-rPrP and ß-rPrP denaturation processes (Figure 2) and observed that the transitions lead to different denatured states (U and U'), which appear to arise from different folding routes: α-rPrP denatures into U with smaller changes in volume whereas ß-rPrP denatures into U' with a larger volume change. There is a clear kinetic barrier, both in the volume (activation volume) and in the Gibbs free energy (activation energy) between U and U' (Figure 2). This unusual property is probably related to both the slow *in vivo* conversion and to the infectious nature of prion diseases. It may also explain the inability to show that any ß-sheet-rich form obtained from recombinant PrP is an efficient infectious agent. Nevertheless, *in vitro* ß-sheet isoforms have physical properties similar to PrP^{Sc}, and amyloid-like aggregates exhibit epitopes equivalent to those of

scrapie PrP (26). Indeed, it is quite intriguing why infectious PrP^{Sc} cannot be refolded *in vitro*, without a PrP^{Sc} template, as demonstrated by Kocisko et al. (57).

The greater pressure stability of α-rPrP, as determined by its higher standard Gibbs free energy change of unfolding ($\Delta G^0 = 5.37 \pm 0.15$ kcal/mol) in contrast to that of ß-rPrP ($\Delta G^0 = 2.81 \pm 0.10$ kcal/mol), seems paradoxical at first glance since the chemical potential of these two forms shows the opposite. For the free-energy diagram, we assumed a metastability model for the conversion of α-rPrP into ß-rPrP (25). The apparent contradiction is resolved by the finding that their N ↔ U transitions are not connected at equilibrium, and by the fact that α-rPrP is converted into ß-rPrP by increasing the temperature and no further unfolding is caused by temperatures as high as 95ºC. The volume and free-energy diagrams revealed by pressure agree with recent thermodynamic and kinetic data showing that partially structured intermediates are essential in the folding pathway (52,58,59).

Another interesting finding was that pressure could distinguish between early and late prion aggregates obtained by incubation at high temperatures for short or long time. The rPrP aggregate obtained by incubation at 50ºC for 2 days was completely pressure resistant, in contrast with the aggregate incubated for 2 h at the same temperature. This was rather surprising because, generally, oligomeric proteins (37,60) and amyloid aggregates (38,44) dissociate in the pressure range from 100 to 300 MPa. And, according to the principle of Le Châtelier, pressure shifts the equilibrium to conformational states that occupy smaller volumes (37,60), normally leading to dissociation of oligomeric proteins (35) or to protein unfolding (60). However, since this mature aggregate did not unfold up to 1,200 MPa, we assumed that it no longer contained internal cavities susceptible to pressurization, and hence could be considered rather densely packed.

Another interesting application of pressure in the PrP studies is to try to obtain PrP aggregates, which could be used as models for the prion scrapie. The rPrP from hamster was converted to a novel misfolded conformer that aggregated in amyloid fibrils by overnight incubation at 600 MPa, 40ºC (32). And these pressure-induced aggregates were also resistant to proteinase K.

In conclusion, we believe that high pressure is a valuable tool for investigating prion transition even without the concomitant use of temperature or chemical denaturants, and recombinant prion aggregates display different susceptibilities to high pressure depending on time of exposure to high temperature during aggregation. We show that different folded conformations as well as different denatured states of rPrP can be distinguished on the basis of hydration, surface exposure and cavities. These dissimilarities result in the paradox that ß-rPrP is highly resistant to temperature whereas it is very sensitive to pressure; the opposite occurs with the native α-rPrP.

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