Pressure–temperature folding landscape in proteins involved in neurodegenerative diseases and cancer

ARTICLE *in* BIOPHYSICAL CHEMISTRY · JUNE 2013

Impact Factor: 1.99 · DOI: 10.1016/j.bpc.2013.06.002 · Source: PubMed

3 AUTHORS:

Yraima Cordeiro
Federal University of Rio de Janeiro
69 PUBLICATIONS **1,270** CITATIONS
SEE PROFILE

Debora Foguel
Federal University of Rio de Janeiro
99 PUBLICATIONS **2,705** CITATIONS
SEE PROFILE

Jerson L Silva
Federal University of Rio de Janeiro
181 PUBLICATIONS **5,133** CITATIONS
SEE PROFILE

Biophysical Chemistry xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Review

Pressure–temperature folding landscape in proteins involved in neurodegenerative diseases and cancer ☆

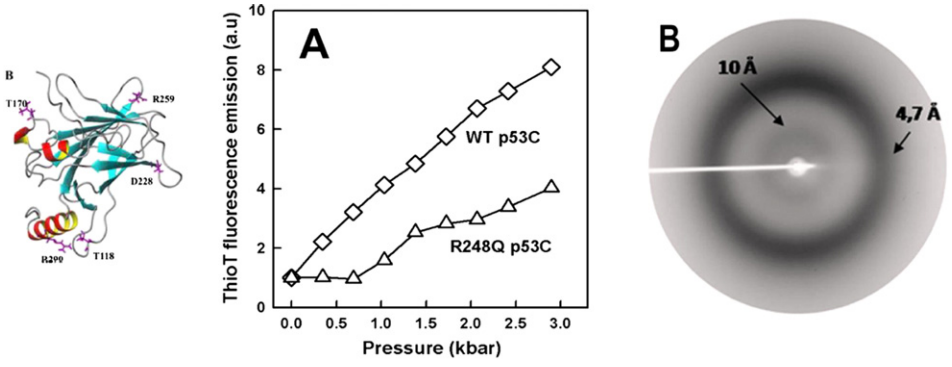
Yraima Cordeiro [a], Debora Foguel [b], Jerson L. Silva [b,*]

[a] Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, Rio de Janeiro 21941-902, Brazil
[b] Instituto de Bioquímica Médica, Instituto Nacional de Biologia Estrutural e Bioimagem, Universidade Federal do Rio de Janeiro, Rio de Janeiro 21941-590, Brazil

HIGHLIGHTS

- High hydrostatic pressure (HHP) is a useful tool to study protein misfolding and protein aggregation reactions.
- HHP has been employed to study misfolding and aggregation of p53, prion proteins, transthyretin and α-synuclein.
- HHP can be used in the high-throughput screening of compounds that affect the misfolding of proteins involved in diseases.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 27 April 2013
Received in revised form 3 June 2013
Accepted 3 June 2013
Available online xxxx

Keywords:
High pressure
Protein misfolding
Protein aggregation
Amyloid
Degenerative disease

ABSTRACT

High hydrostatic pressure (HHP) is a valuable tool to study processes such as protein folding, protein hydration and protein–protein interactions. HHP is a nondestructive technique because it reversibly affects internal cavities excluded from the solvent present in the hydrophobic core of proteins. HHP allows the solvation of buried amino acid side chains, thus shifting the equilibrium towards states of the studied molecule or molecular ensemble that occupy smaller volumes. HHP has long been used to dissociate multimeric proteins and protein aggregates and allows investigation of intermediate folding states, some of which are formed by proteins involved in human degenerative diseases, such as spongiform encephalopathies and Parkinson's disease, as well as cancer. When coupled with nuclear magnetic resonance and spectroscopic methods such as infrared and fluorescence spectroscopy, HHP treatment facilitates the understanding of protein folding and misfolding processes; the latter is related to protein aggregation into amyloid or amorphous species. In this review, we will address how HHP provides information about intermediate folding

Abbreviations: ANS, 1-anilino 8-naphthalene sulfonate; α-syn, alpha-synuclein; bis-ANS, 4, 4′-bis(1-anilinonaphthalene 8-sulfonate); DA, dopamine; FTIR, Fourier transform infrared; HHP, high hydrostatic pressure; I state, intermediate state; LB, Lewy bodies; NMR, nuclear magnetic resonance; p53, p53 tumor suppressor protein; PD, Parkinson's disease; PrP, prion protein; rPrP, recombinant prion protein; TEM, transmission electron microscopy; TTR, transthyretin.

* Corresponding author at: Instituto de Bioquímica Médica, Instituto Nacional de Biologia Estrutural e Bioimagem, Universidade Federal do Rio de Janeiro, Av. Carlos Chagas Filho 373, bloco E, sala 10, Rio de Janeiro 21941-590, Brazil.
E-mail address: jerson@bioqmed.ufrj.br (J.L. Silva).

0301-4622/$ – see front matter
http://dx.doi.org/10.1016/j.bpc.2013.06.002

states and the aggregation processes of p53, which is related to cancer, and prion proteins, transthyretin and α-synuclein, which are related to human degenerative diseases.

Contents

1. Introduction

Pressure is a thermodynamic variable with longstanding successful biological applications [reviewed in 1–5]. However, the full potential of high-pressure methods has not been assessed. The seminal article by Bridgman in 1914 [6], in which he observed the pressure-mediated denaturation of ovalbumin, introduced the promising properties of the pressure approach. In the last 50 years, several groups have used pressure to explore protein denaturation, dissociation, and conformational changes [see reviews: 1–3,7–9].

The advantage of using pressure to observe the equilibrium of protein conformation is the simplicity of the data analysis. For a given change produced by pressure in a protein P,

$$P \leftrightharpoons P',$$

and pressure will drive the equilibrium according to the formula:

$$d\Delta G(T)/dp = \Delta V(T), \quad (1)$$

where ΔV represents the difference between the volumes occupied by the solvated products (P′) and reactants (P). The equilibrium constant K(p) can be expressed by

$$d\ln K(p)/dp = -\Delta V(p)/RT. \quad (2)$$

HHP has provided promising results on protein denaturation and dissociation, as well as the folding and stability of protein–DNA complexes, virus particles and amorphous and fibrillar aggregates [1,2,10,11]. Pressure changes the equilibrium in the direction of the species that occupies the lower molar volume. Because of the large number of atoms involved, packing defects cannot be avoided, and these defects lead to formation of cavities and void volumes inside the native fold that render proteins susceptible to HHP [12,13].

2. Squeezing amyloid fibrils

One of the most challenging questions in biology is how proteins find their unique set of native-state conformations based only on the information coded by their primary sequences (self-assembly) [14,15]. Incompletely folded chains that form en route expose regions that are buried in the native state [16–18]. Such species are prone to inappropriate contacts with neighboring molecules, giving rise to protein aggregates that exert their effects either by a "loss of function" (lack of active protein) or by a "toxic gain of function" (cytotoxicity of aggregates) [reviewed in: 19]. Living systems have evolved elaborate strategies to minimize this problem, including molecular chaperones and folding catalysts, as well as a stringent "quality control" mechanism that operates in the ER and a "clearance machine" for protein degradation (the ubiquitin–proteasome system) [20,21]. Unfortunately, despite these "housekeeping" strategies, under certain circumstances (e.g., stress, protein turnover, natural protein unfolding inside cells, and aging), aggregation occurs and can cause disease [22,23]. In amyloidogenic diseases, aggregates have a common organized architecture, known as the amyloid fibril [reviewed in: 18]. Alzheimer's disease, Parkinson's disease and approximately 40 other misfolding disorders are examples of these diseases. The amyloid fibril has a cross-β fold [24]. Recently, some atomic structures of amyloid fibrils have been solved [25–27]. There is abundant evidence that the interface formed by the contact between two β-sheets in the fibril is dry (lacking water molecules), while the outer faces are wet. The ability to form amyloid fibrils is not restricted to proteins involved in amyloidogenic diseases, but it seems to be a general propensity of all polypeptide chains [28]. In most cases, protein aggregation proceeds when a transient intermediate (I) conformation builds up during folding or unfolding. The accepted idea concerning the tendency of the I state to aggregate is based on its low solubility due to the presence of a large, hydrophobic surface that is still exposed to the aqueous environment [29]. This surface creates conditions for inter- and intra-subunit contacts. These species are formed under certain stress conditions during the unfolding or processing of a protein, either in a cellular compartment or in the extracellular space [30].

In vitro, chemical or physical tools are used to study these partially folded conformations and allow their structural characterization. High hydrostatic pressure (HHP) is a powerful method to trap such conformations [4]. Partially folded conformations are more "open" structures (fewer solvent-excluded cavities, lower molar volume), which might explain why they are favored under high pressure. In fact, intermediate states are not stabilized by pressure, they are just less destabilized than folded states; therefore, under pressure, I states are only relatively more stable than the native state (allowing them to be studied by applying HHP). HHP leads generally to a decrease in the length of H-bonds, but not their rupture, which explains why at least some of the protein secondary structure can be preserved under pressure.

Some amyloid fibrils seem to present cavity defects because they are dissociated by relatively low pressure [31–37]. However, mature amyloids in general possess a strong hydrogen bonding network that maintains the fibrils tightly packed, reducing their susceptibility to HHP. Amyloid fibril formation evolves through the population of species with different packing, morphology, number of subunits, and H-bonding stabilization. Therefore, it is reasonable to visualize HHP as a tool that allows differentiating

Please cite this article as: Y. Cordeiro, et al., Pressure–temperature folding landscape in proteins involved in neurodegenerative diseases and cancer, Biophysical Chemistry (2013), http://dx.doi.org/10.1016/j.bpc.2013.06.002

between early and late aggregates, and to elucidate the interactions that stabilize such intermediate states that occur along the formation of a mature fibril. Spectroscopic properties that can be measured under pressure (e.g., fluorescence, absorption, FTIR, Raman, NMR) can provide details of partially folded states. As will be further reviewed here, the use of HHP with spectroscopic and structural tools has allowed the dissection of the role of packing and cavities in protein misfolding and aggregation.

Herein, we will concentrate on four amyloidogenic proteins: 1) prion protein (PrP), which is involved in transmissible spongiform encephalopathies (TSEs); 2) tumor suppressor protein p53, which is related to cancer; 3) transthyretin (TTR), which is involved in senile systemic amyloidosis (the wild-type protein), cardiomyopathy, central nervous system deposits and familial amyloidotic polyneuropathy (caused by more than 90 TTR variants); and 4) α-synuclein (α-syn), which is associated with Parkinson's disease (both wild-type and variants).

3. HHP and PrP: insights into structural conversion, stability and aggregation

PrP is a partially unfolded protein that is involved in neurodegenerative diseases, and its conformation and stability have been widely investigated in the past 30 years. The importance of PrP as the main agent responsible for TSEs is unquestioned [38,39], although other molecules, such as glycosaminoglycans, lipids and nucleic acids, have been implicated in the conversion of normal cellular PrP into an abnormal conformation, named PrP^{Sc} [40–42]. The conversion of PrP^{C} into PrP^{Sc} involves the acquisition of a higher β-sheet content in the latter form, and diminishes PrP solubility in aqueous solvents [43]. To understand the conversion process and why, once formed, PrP^{Sc} triggers the misfolding of native PrP present in the affected organism, a set of biophysical data was obtained on the stability of PrP. The PrP primary sequence is formed by 209 amino acid residues, and native PrP has two structurally distinct domains: an N-terminal flexible region (residues 23 to ~125) and an α-helical C-terminal globular domain (~125 to 231) [44], that are highly conserved among different species [45] (Fig. 1A). One of the properties that distinguishes PrPs from different species is the charge distribution on the protein surface (Fig. 1B) [46]. Charge distribution and cavities likely affect stability under conditions of hydrostatic pressure. The cross-talk between these different PrP domains also warrants investigation because conversion into PrP^{Sc} is reflected by secondary structure changes, mainly in the N-terminal region (residues 90 to ~140) [47] but can also affect the C-terminal domain [48,49]. Most of the biophysical data were collected with different recombinant mammalian PrPs, either mature full-length PrPs, comprising residues 23–231 or 23–230 or truncated versions of PrP, mainly containing the globular C-terminal domain. Herein, we will focus on the application of HHP combined or not combined with high temperature on native and aggregated recombinant PrP (rPrP) forms and how these results contribute to our understanding of PrP stability and the generation of scrapie-like conformations.

Incubation of rPrP at temperatures above 40 °C leads to PrP unfolding and aggregation into amorphous species [9,35,50]. These aggregates have increased β-sheet content as revealed by Fourier transform infrared (FTIR) spectroscopy [35,50]. HHP was applied to dissociate temperature-induced aggregates of Syrian hamster rPrP (globular domain, residues 90–231) [50] and full-length murine rPrP [35] (Fig. 2). Dissociation curves provide us with the thermodynamic parameters for β-sheet-rich PrP unfolding, which is an alternative conformation of PrP that is possibly related to PrP^{Sc} (at least in secondary structure content). Pressure jumps were applied to thermal-aggregated Syrian hamster PrP (ShaPrP), and pressure values of ~2 kbar were sufficient to maintain ShaPrP in the native state, even at temperatures >70 °C [50]. This approach was also applied to amyloid fibrils of hen lysozyme that was deficient in disulfide bonds and thus prone to aggregation [51]. Rapid pressure increase (~1 min) up to 2 kbar dissociated preformed fibrils into monomers; this process was fully reversible [51]. Such thermal-induced aggregates are not densely packed because they are susceptible to dissociation by relatively mild HHP values (~2 kbar). Moreover, these aggregates bind the fluorescent probes 1-anilino 8-naphthalene sulfonate (ANS) and 4,4′-bis(1-anilinonaphthalene 8-sulfonate) (bis-ANS), which interact with protein cavities and do not bind to completely unfolded or packed species [52–54]. The formation of rPrP aggregated species is kinetically driven as the conformation and packing of these aggregates change over time. When rPrP23–231 is incubated at 50 °C for 48 h,

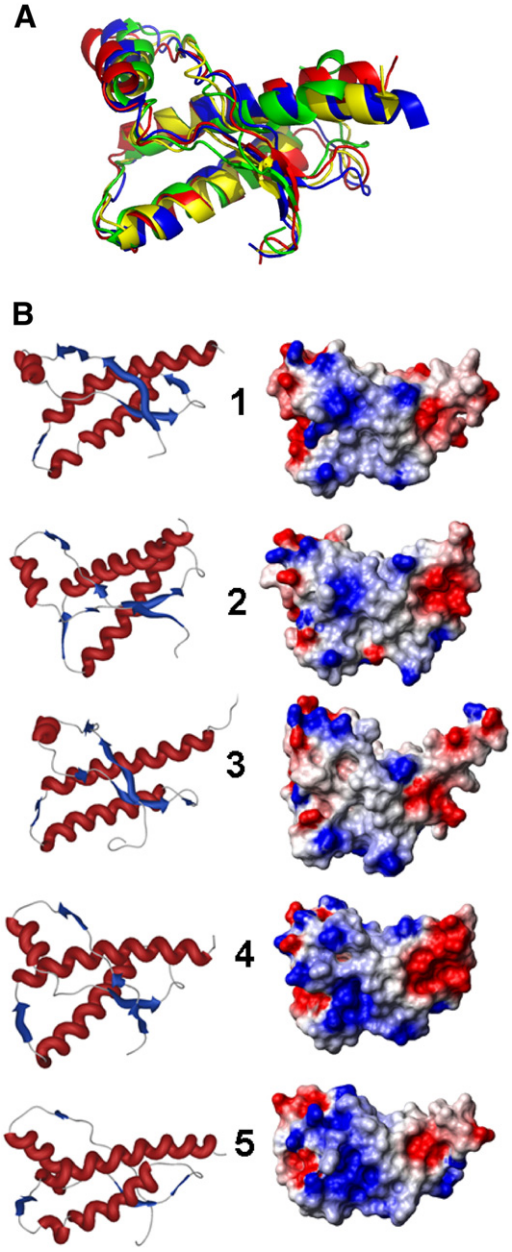


Fig. 1. NMR structures of mammalian prion proteins and charge distribution in the protein surface. A, Superposition of the globular domains of recombinant PrP from cattle (1DX0.pdb) (red), mouse (1AG2.pdb) (green), human (1QLX.pdb) (blue), and Syrian hamster (1B10.pdb) (yellow). Figure was prepared with PyMOL. B, (1) human PrP, 1QM1.pdb; (2) cattle PrP, 1DWZ.pdb; (3) mouse PrP, 1XYX.pdb; (4) Syrian hamster PrP, 1B10.pdb; (5) rabbit PrP, 2FJ3.pdb. Left: Ribbon representations of the secondary and tertiary structures of the PrPs. Right: Distributions of electrostatic potentials (blue: positive charges; red: negative charges).
Modified from reference [46].

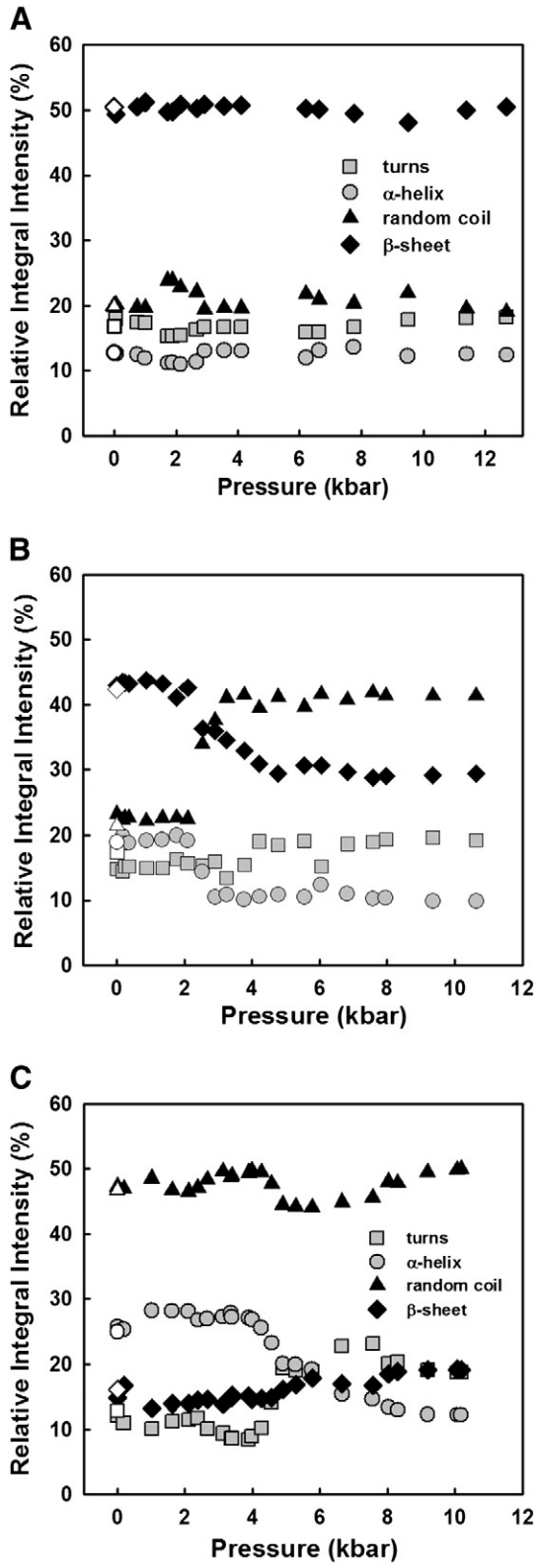


Fig. 2. Pressure effects on murine full-length rPrP (rPrP23–231) after different treatments, investigated by HHP-FTIR. Pressure susceptibility of rPrP aggregated at 50 °C for 48 h (A) or 2 h (B), and native rPrP at 25 °C (C). Secondary structure components are shown after deconvolution and peak fitting of the amide I region of FTIR spectra. Figure adapted from [35].

the resultant oligomer is uncompressible, even at 12 kbar [35] (Fig. 2A), while incubation at the same temperature for 2 h resulted in pressure-sensitive aggregates (Fig. 2B). This result suggests the reorganization of aggregates and the loss of internal cavities over time.

HHP-FTIR was used to follow secondary structure changes in full-length murine rPrP (rPrP23–231) and in truncations lacking portions of the N-terminal domain [35,55]. This approach allows an appreciation of the role of the flexible N-terminal domain in stability under HHP conditions and in the aggregation of rPrP induced by HHP and temperature. FTIR analysis revealed that although the rPrP globular domain is highly stable under pressure, this protein is partially unfolded above 5 kbar, with a loss of α-helices accompanied by an increase in random coils and turns [35] (Fig. 2C). The reversibility of this effect allows the calculation of the thermodynamic parameters of unfolding (Table 1). HHP-FTIR measurements revealed that temperature-induced rPrP β-sheet aggregates (scrapie-like) are less stable than a native α-helical rPrP [35] (Fig. 2). Moreover, this stability seems to depend on the presence of the N-terminal domain, as rPrP variants lacking the octarepeat region (residues 50–91) and residues 32–121 are more prone to aggregation after pressurization than full-length rPrP [55]. Another report showed that rPrP aggregates as amyloid fibrils after pressure treatment, even without the N-terminal domain [53]. Protofibrils and globular aggregates that are resistant to proteinase K (PK) treatment form after a return to atmospheric pressure and are characterized by thioflavin T (ThioT) binding [53]. The time of pressurization is important to define the aggregate morphology because amyloid fibrils form after only overnight incubation at 6 kbar [53].

Although we verified that the N-terminal domain increases rPrP stability against HHP-induced unfolding [55], the importance of the N-terminal region of rPrP in driving aggregation and in overall rPrP stability remains controversial. The combined use of pressure, temperature and pH can aid in our understanding of the importance of each PrP domain in protein aggregation and conformational conversion. Additionally, the presence of different folding intermediates that may be populated in vivo can be revealed. High-pressure NMR studies on the full-length and globular domain (121–230) truncated variant of human rPrP (huPrP) showed that HHP could reveal structurally similar folding intermediates for both variants [56]. In this case, the N-terminal region did not dictate a propensity for conformational change, but transient interactions of C-terminal residues with the flexible domain were identified [56]. As expected (Fig. 1), similar conformational states for the globular domains of huPrP and ShaPrP were revealed by HHP coupled with NMR analysis, with the identification of two intermediate states for each protein [57]. However, specific residues in huPrP and ShaPrP display different responses to pressure (up to 2.5 kbar), yielding different ΔG_0 values

Table 1
Thermodynamic parameters calculated from pressure studies with different prion protein constructions.

Protein model	Temp. (°C)	ΔG (kcal/mol)	ΔV (ml/mol)	Reference
mPrP23–231 (native)	25	5.37 ± 0.15	−29	[35]
mPrP23–231 (β-sheet, aggregated)	50	2.81 ± 0.10	−43.6 ± 7	[35]
ShaPrP90–231 (non-aggregated)	40	0.94[a]	−31.9 ± 1.9	[50]
huPrP121–230 (native)	20	4.44 ± 0.69[b]	−125 ± 32[b]	[56]
ShaPrP90–231 (native)	30	5.54 ± 0.84[b]	N.D.	[57]
mPrP23–231 (amyloid fibril)	25	8.86 ± 0.31[c]	−18.3 ± 1.7[c]	[37]
mPrP23–231 (amyloid fibril)	25	9.63 ± 0.31[d]	−23.8 ± 1.8[d]	[37]

All ΔG values were calculated assuming two state transitions. [a]$\Delta G^{U}_{2.0\ kbar}$, 40 °C; [b]free energy and volume changes calculated from transition of native to intermediate states of PrPs. Free energy and volume changes obtained for the fast[c] and slow[d] phases of mPrP amyloid fibril dissociation by fast pressure increase to 6 kbar; N.D., not determined.

Please cite this article as: Y. Cordeiro, et al., Pressure–temperature folding landscape in proteins involved in neurodegenerative diseases and cancer, Biophysical Chemistry (2013), http://dx.doi.org/10.1016/j.bpc.2013.06.002

[57] (Fig. 3). These small but significant differences might be related to the prion species barrier. HHP populates excited protein states that can be detected by NMR, even if they only represent a minor fraction of the total protein conformation in solution. Following pressure-induced chemical shift variations, one can identify conformational changes in every amino acid residue from the studied protein. A metastable intermediate state of ShaPrP90–231 was identified by 2D NMR coupled with pressure variation at low pH [58]. Although it represents only a small fraction of the total ShaPrP in solution, this intermediate state might be a precursor to PrP amyloidogenesis.

HHP treatment can dissociate preformed temperature-induced aggregates [9]. However, HHP can also induce protein aggregation, as it can stabilize misfolded intermediate species (in relation to the native conformation) that are prone to aggregation. These species can aggregate even under HHP or after decompression to atmospheric pressure [9,59]. Transmission electron microscopy (TEM) and spectroscopic analysis of pressure-induced PrP aggregates showed that, depending on the pressure applied, pH values and time of pressurization, different supramolecular structures are populated [54]. Pressurization at 6.0 and 4.7 kbar at basic and neutral pH induces the formation of aggregated (Agg_{HP}) and intermediate (I_{HP}) species, respectively, of full-length murine rPrP [54]. Immediate return to atmospheric pressure leads to the conversion of Agg_{HP} into amorphous aggregates and recovers native rPrP for I_{HP}. If pressure is maintained for a longer period (0.75 d for Agg_{HP} and 8 d for I_{HP}), spherical and fibrillar aggregates form, suggesting different aggregation pathways for this protein that are kinetically driven and dependent on the sample environment. ANS binds mainly to the amorphous aggregates, with increased fluorescence emission compared to fibrillar and spherical aggregates and the native protein [54]; thus, packing differences for pressure-induced aggregates are evident. It remains to be established whether these aggregated species present different toxicity levels to cells that are susceptible to prion infection. Similarly, HHP and pressure jumps were applied to toxic amyloid fibrils of murine rPrP, resulting in irreversible disaggregation and reduced toxicity to primary neuronal cells [37]. The kinetics of amyloid fibril dissociation was followed by thioflavin T binding, and thermodynamic activation parameters were obtained. Both activation entropy and enthalpy seemed to control the reaction kinetics induced by pressure, and a high-energy barrier was responsible for the high stability of amyloid fibrils to pressure [37]. In Table 1 we list the thermodynamic parameters obtained for different PrP constructions under HHP and/or temperature treatment.

Another application of HHP is to diminish prion infectivity in brain or food samples. HHP coupled with high temperature reduces the infectivity of the 263K prion strain and renders PrP^{Sc} extracted from infected brains susceptible to PK digestion [60]. Additionally, treatment of sausages spiked with the same prion strain with HHP at 12 kbar and 135 °C reduces prion infectivity (measured as LD_{50}) by a factor of 10^6 [61].

In general, pressure treatment changes the physicochemical nature of PrP and other proteins. As the native state is less pressure-stable than partially folded states, HHP allows to trap different I states that, upon decompression, can aggregate into distinct species with different packing and morphologies. HHP has provided quantitative information about the stability of different mammalian PrPs (Table 1) and has helped to reveal PrP misfolded intermediates, dissociate preformed aggregates, reduce prion infectivity in biological samples and trigger PrP aggregation into potential scrapie-like forms or precursors.

4. Cancer, amyloid, and p53

p53 is involved in cell cycle control, and its malfunction contributes to diverse types of cancer [62,63]. This protein is organized as a tetramer, each subunit composed of a central DNA-binding domain, an N-terminal region responsible for transcriptional activation and a C-terminal domain that maintains tetramerization [64]. p53 binds several protein targets [62] in the cell, and both wild-type p53 and hot-spot mutants can aggregate in vitro and in vivo [62,65–73]. Some of these p53 aggregates possess an amyloid nature [66,69–71,73], suggesting that cancer might share some similarities with protein conformational diseases.

When incubated at temperatures above 37 °C, the central domain of WT p53 (p53C) and of a p53 hot-spot mutant (R248Q) undergoes unfolding and oligomerization, as verified by light scattering and intrinsic fluorescence [65,66]. HHP treatment (up to ~3 kbar) at 37 °C, also leads into p53 aggregation, but the morphologies of temperature-induced and pressure-induced aggregates are significantly different [66,74]. While high temperature treatment induces the predominant formation of amorphous aggregates, HHP generates annular aggregates and amyloid-like fibrils, as observed by atomic force microscopy, TEM and ThioT binding [66]. The morphology of the aggregates changes with time after a return

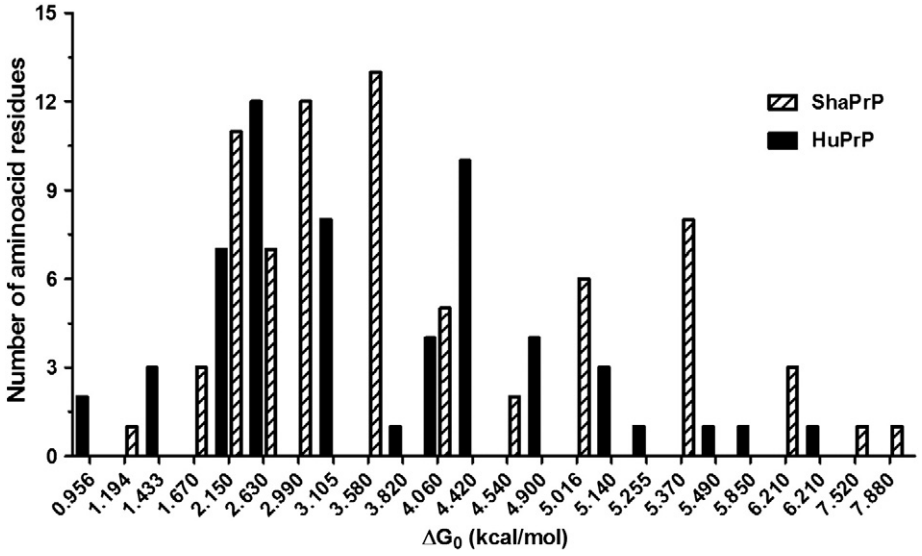


Fig. 3. Different HHP susceptibilities of specific residues of mammalian prion proteins. ΔG_0 values at atmospheric pressure obtained after HHP treatment of the globular domains (121–231) of human (huPrP) and hamster (ShaPrP) prion proteins. Free energy values were calculated for individual residues. Depicted residues are distributed mainly along loop L2, α-helix 1, the second half of α-helix 2, and α-helix 3.
Adapted from [57].

Please cite this article as: Y. Cordeiro, et al., Pressure–temperature folding landscape in proteins involved in neurodegenerative diseases and cancer, Biophysical Chemistry (2013), http://dx.doi.org/10.1016/j.bpc.2013.06.002

to atmospheric pressure, as fibrillar aggregates are only visualized ~1 month after decompression [66]. Ishimaru et al. [66] used HHP as a mild tool at neutral pH to obtain ordered aggregation of WT or R248Q p53C. The formation of amyloid-like fibrils suggested that cancer could be caused by misfolded p53 that is sequestered into high-molecular-weight aggregates, thus losing its function. Investigation of the same p53C constructs at low pH (5.0) revealed that they adopt a molten globule conformation with a residual secondary structure content and higher hydrodynamic radius compared to their native counterparts (pH 7.2) [75,76]. Both WT p53C and R248Q p53C are less stable under pressure treatment at pH 5.0 than at pH 7.2, indicating that a reduction in pH, as occurs in tumor environments [77], favors its loss of function. X-ray diffraction analysis, Thio-T binding, Congo red binding and TEM confirmed that oligomeric species and amyloid fibrils of p53 form after pressure (Fig. 4) and temperature treatment at pH 7.2 [71]. These aggregates are toxic to mammalian cells in culture, and aggregated R248Q p53C seeds wild-type p53 aggregation [71], characteristics that are typical of prions. The initial pressure studies showing the amyloid character of p53 aggregation resulted in studies to test whether mutant p53 would act as a prion [66,68,71]. The prion-like behavior of oncogenic p53 mutants provides an explanation for their negative dominance effect and indicates that they may serve as a potential target for cancer therapy [71].

We also found that protein binding to a consensus DNA sequence increases p53C stability under pressure conditions and prevents the formation of the misfolded conformation, an effect that is related to the DNA-binding affinity [67]. Interference with the DNA-binding affinity by altering the ionic strength either increases or completely abolishes the observed stability of the protein. The finding that full-length p53 is dramatically stabilized by the binding of a cognate sequence is of particular biological and medical interest. This increase in DNA-mediated protein stability might be relevant for therapeutic approaches based on strategies for the rescue of misfolded wild-type p53C or denatured p53C mutants. Additionally, cognate DNA is capable of rescuing most of the native conformation of a misfolded protein [67].

In summary, HHP and temperature treatments have been used to change the conformation of p53 into different aggregates and molten globule states.

5. TTR

TTR is a homotetrameric protein that carries retinol binding protein and thyroxin in the plasma [78,79]. Misfolding and aggregation of both wild-type and mutants of TTR cause human amyloidogenic diseases, such as senile systemic amyloidosis for wild-type TTR and familial amyloidotic polyneuropathy (FAP) and familial amyloidotic cardiomyopathy for unstable TTR mutants [79–81].

Treatment of TTR with high pressure leads to partially unfolded monomers; upon a decrease in pressure, altered tetramers form with weaker subunit interactions and amyloidogenic properties [31–33]. These altered monomers and tetramers have been studied using fluorescent dyes at equilibrium. At high pressure, the tryptophan fluorescence emission of TTR exhibits a large red shift, indicating substantial denaturation, but the protein can still bind bis-ANS, suggesting the persistence of tertiary contacts [31]. The most surprising result from these studies was the observation of fibril formation immediately after decompression in relatively mild pH and temperature conditions (pH 5.0; 37 °C; 1–3 μM TTR) (Fig. 5A). The thermodynamic stability of wild-type TTR has been compared with that of aggressive amyloidogenic mutants (L55P and V30M) and with the non-amyloidogenic mutant T119M [32].

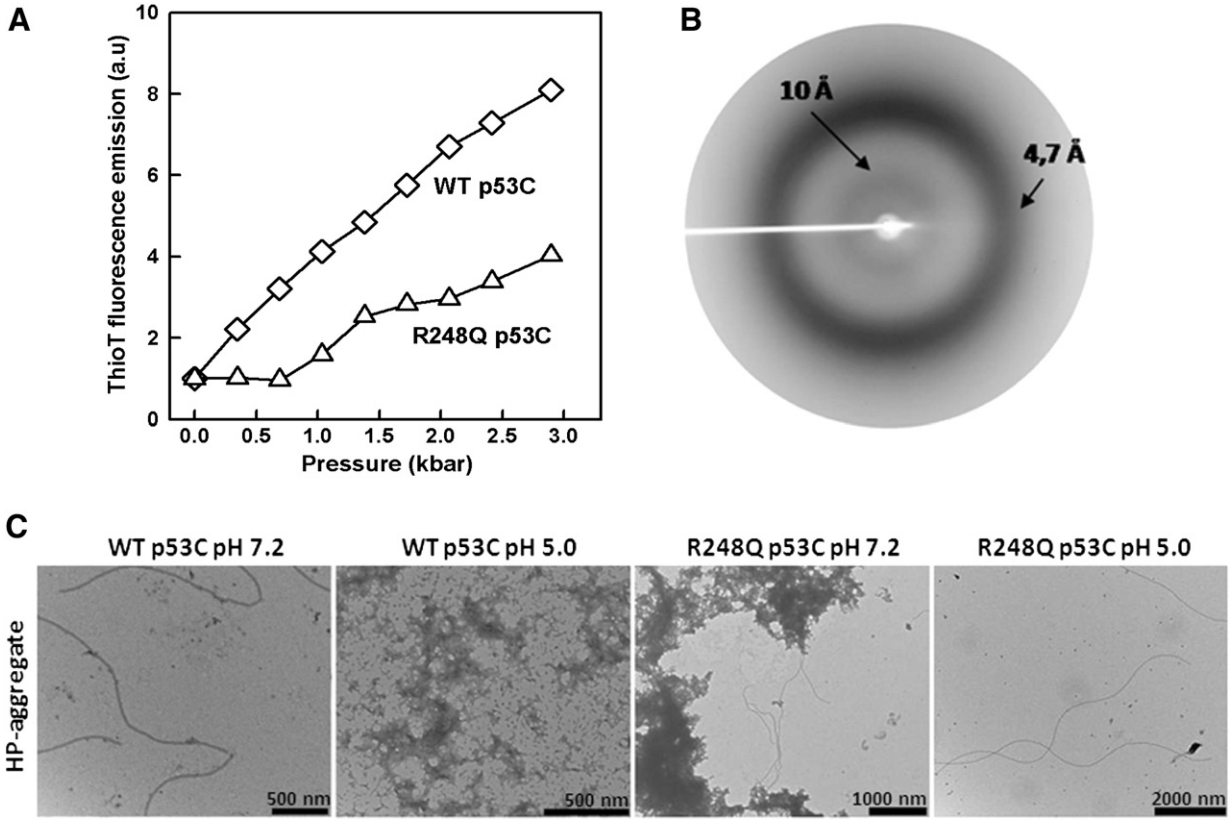


Fig. 4. HHP-induced aggregation of WT and R248Q p53 central domains. A, ThioT fluorescence emission area (50 μM) as a function of the pressure increase for WT (diamond) or R248Q (triangle) p53C (5 μM) at pH 7.2. B, X-ray diffraction spectrum of R248Q p53C after HHP-induced aggregation at pH 7.2. C, TEM images of HP aggregates of WT and R248Q p53C at pH 7.2 and pH 5.0. HP aggregates were formed by incubation of samples at 5 μM with pressures up to 3 kbar at 37 °C.
Figure adapted from [71].

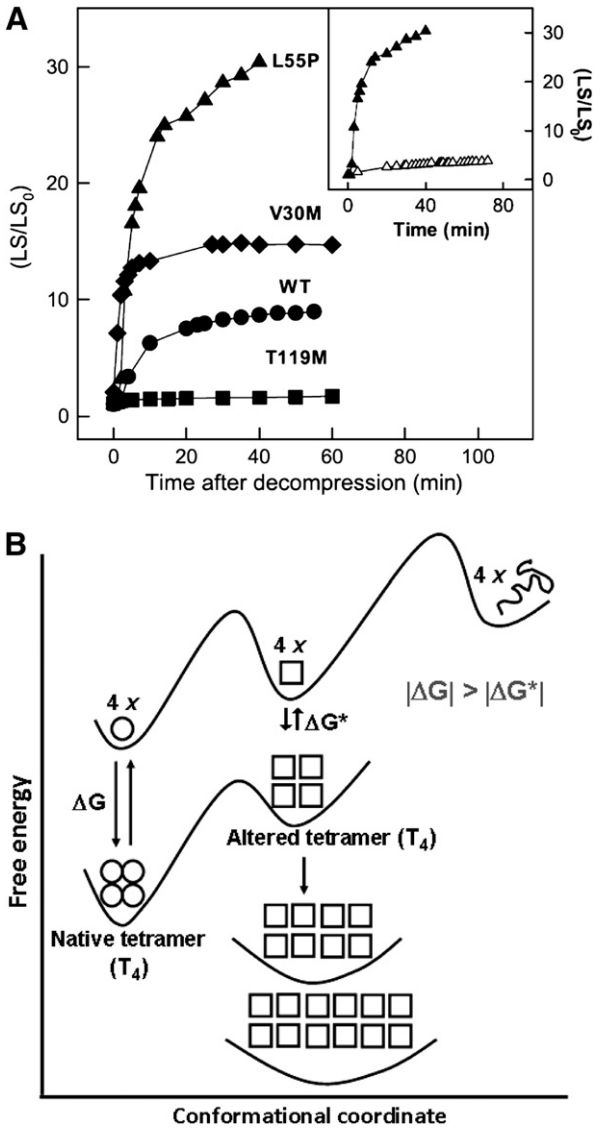


Fig. 5. HHP effects on TTR: population of an altered tetramer prone to aggregation. A, wild-type and mutant TTR [(■) T119M, 5 μM, pH 3.7; (●) wt, (♦) V30M; (▲) L55P] subjected to 3 kbar for ~60 min at 37 °C, pH 5.6. Light-scattering (LS) was measured after pressure release (relative LS values are shown). Inset: aggressive mutant, (△) L55P was incubated at pH 5.6 at 1 atm and LS was recorded; (▲) curve obtained after pressure release (panel obtained from reference [32]). B, Free-energy diagram for TTR dissociation, unfolding, and aggregation. Circles: native tetramer and monomer; squares: altered tetramer, monomer, and aggregates; line: denatured, unfolded monomer.
Modified from reference [31].

Amyloidogenesis and thermodynamic stability are negatively correlated, where L55P is the least stable variant and has the highest propensity for fibrillogenesis [32] (Fig. 5A).

The oligomeric state of TTR that gives rise to these fibrils is a "loose" tetramer that is found in equilibrium with a small fraction of monomers, as visualized by gel-filtration chromatography [31]. This altered tetramer, called T_4^* (pre-aggregated state), is in equilibrium with the monomeric species, which is also able to aggregate into fibrils. The T_4^* state appears to be physiologically relevant for two reasons: it seeds aggregation, and it can act as a reservoir of altered monomers that might rapidly aggregate (Fig. 5B). The T_4^* state could be prevented to aggregate by keeping it at low temperature (4 °C). In this respect, amyloid aggregation reaction is very similar to other association reactions such as virus assembly [82].

Another potential use of HHP is for drug screening. Because TTR and other amyloidogenic proteins aggregate within a few minutes after decompression (~30 min), in a single day it is possible to scan several compounds with the potential to inhibit fibril formation [33,83]. In the case of TTR, this represents an enormous improvement because under optimal conditions at atmospheric pressure, TTR aggregation takes 72 h to complete.

More recently, HHP has been employed to explore potential therapeutic strategies against TTR amyloidogenic diseases by trapping the monomer of a non-amyloidogenic variant of TTR [84]. The combination of HHP with cold temperature and low urea concentration produced long-lived monomers of the non-amyloidogenic mutant T119M. When they were mixed with aggressive TTR mutants, non-amyloidogenic heterotetramers were generated [84]. These results underscore the potential of HHP to help us understand and treat amyloidogenic diseases.

6. Pressure studies on α-synuclein

Parkinson's disease (PD) is one of the major neurodegenerative disorders of middle and old age; it is characterized by muscle rigidity, tremor and bradykinesia, with dementia occurring in a minority of patients [85,86]. The main pathological hallmarks of PD are characteristic inclusions called Lewy bodies (LB) that occur in the cytoplasm of neurons, predominantly in the *substantia nigra* [86–88]. The major component of LB is α-syn, a soluble unfolded protein that can aggregate into insoluble amyloid fibrils that then form LB, followed by ubiquitination and accumulation of neurofilaments [86,87]. The healthy neurons in PD patients are the ones that accumulate LB; this finding has led researchers to question whether LB are the cause of the symptoms of PD or a cellular strategy to eliminate α-syn and other neurotoxic molecules [85–87,89]. Natural mutants of α-syn (A53T and A30P) have been identified in families with early onset PD [90,91]. However, the causes of aggregation of α-syn in sporadic forms of PD remain as open questions. The loss of neurons in the *substantia nigra* results in dopamine (DA) dysfunction, and the current therapy for PD is based on L-DOPA replacement [88].

In vitro aggregation of wt, A53T and A30P α-syn is a slow process and leads to fibrils with similar morphologies [89]. The A53T mutation accelerates aggregation, but the effects of the A30P mutation are unclear; it appears to accelerate the formation of non-fibrillar oligomers, which are assumed to represent pre-fibrillar intermediates [89]. Future strategies to design drugs against PD will be based on the possible neurotoxic effects of these pre-fibrils. We investigated the stability under high pressure of α-syn and TTR fibrils (Fig. 6); our most important findings were two-fold: *i*) amyloid fibrils are sensitive to HHP and are dissociated in the same pressure range as dimers, trimers and tetramers; *ii*) the primary sequence influences fibril stability. In the case of α-syn fibrils, wild type fibrils are more resistant to HHP than variant fibrils. This result has important physiological implications because it suggests that the variant fibrils, which are more labile, would generate smaller toxic species more easily than wild type fibrils. These findings are consistent with the recently described model of the amyloid fibril [25] which showed that the fibril core is dry, rendering the fibrils susceptible to HHP, leading to further hydration of exposed side-chains [68] (Fig. 6B).

Similar to α-syn, a disulfide-deficient mutant of hen lysozyme is intrinsically unfolded and can form amyloid protofibrils and fibrils, which are highly sensitive to pressure [34]. The authors showed the reversible dissociation of multimeric precursors of the amyloid fibrils of this protein. Overall, these studies indicate that hydrophobic interactions play a crucial role in fibril assembly and maintenance. HHP has emerged as a promising tool for perturbing amyloid aggregates and for finding new targets in the development of therapeutic compounds to block fibrillogenesis.

These data stimulated studies on the effects of physiologically and therapeutically relevant molecules to evaluate changes in fibril stability. We used the pressure approach to investigate how dopamine (DA)

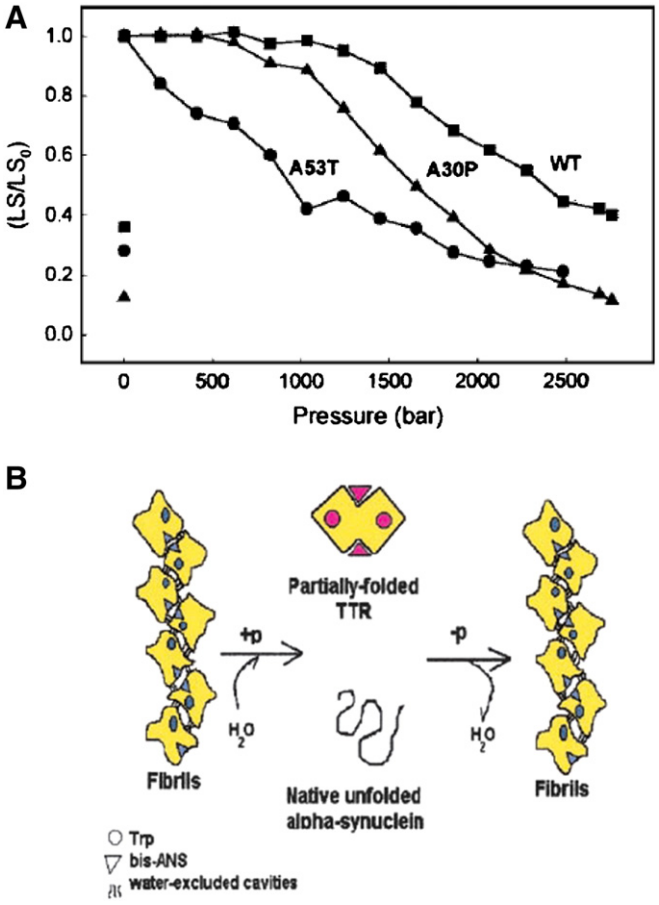


Fig. 6. Fibrils derived from wild-type (wt) proteins (α-syn and TTR) have higher pressure stability than fibrils formed by variant proteins. A, Fibrils from wt or mutant α-syn [wt (squares); A53T (circles); A30P (triangles)] were subjected to increasing pressures at 37 °C and disaggregation was followed by the decrease in light scattering (LS) values. Isolated symbols on the left represent LS values obtained after return to 1 atm. B, Cavities present in the core of the fibrils of wt TTR and α-syn render them susceptible to HHP. Pressure (+p) stabilizes partially folded species (for wt TTR), or native, unfolded monomers (for α-syn), as seen by tryptophan emission and bis-ANS binding assays, allowing void volumes to be replaced by water.
Figure modified from reference [33].

differentially affects the stability of protofibrils and fibrils that were composed of wild-type or α-syn variants (A30P and A53T) [36]. When DA was absent, all α-syn protofibrils exhibited identical levels of stability against HHP; however, in the presence of DA, the variant composed of protofibrils was much more stable. This result implies that α-syn protofibrils last longer, offering a convincing explanation as to why these mutations are so aggressive. When added to mesencephalic and cortical neurons in culture, A30P protofibrils (PFA30P) and A30P DA-protofibrils (DAPFA30P) led to a significant decrease in the number and length of neurites and resulted in more cells undergoing apoptosis. These toxic effects of PFA30P and DAPFA30P were practically abolished by high-pressure treatment, which cracked the protofibrils into smaller aggregates, as visualized by atomic force microscopy [36]. These results suggest that strategies aimed at breaking and/or clearing these aggregates may be promising in alleviating the symptoms of PD.

7. Conclusions and perspectives

Here we reviewed the studies performed in the last 15 years on the effects of pressure on proteins involved in amyloid and protein misfolding diseases. As expected, pressure treatment is a useful but limited approach. In some cases, pressure induces the dissociation of protofibrils and fibrils [4,33,37,51], but it fails to cause dissociation in several cases, as in the dissociation of short peptide segments of TTR [92], β2-microglobulin [93], and islet amyloid polypeptide [94]. In other cases, the population of folding intermediates created by pressure leads to aggregation, e.g., p53 [66]. Several questions remain unsolved. We need to understand how the changes in packing and cavity distribution change as the protein evolves to form small oligomers, protofibrils or protofilaments and, finally, fibrils. Pressure is a strong tool to characterize these different intermediate species [11,33,56,58,95]. This question is especially relevant in neurodegenerative disorders such as Parkinson's disease, prion diseases and Alzheimer's disease, in which the massive destruction of neurons takes place by a toxic gain-of-function. It is usually believed that early pre-fibrillar aggregates can be highly damaging to cells, whereas mature fibrils would be harmless [96–98]. The specific mechanisms that explain these toxic effects are controversial. It is possible that the "doughnut-like aggregates" make pores in membranes, disrupting the ionic balance [96]. It is also possible that such early aggregates, because they have hydrophobic segments that are exposed to the environment, establish aberrant interactions with membranes or other cellular components. All of these interactions must be studied by high-pressure investigations.

The combination of pressure with structural and spectroscopic tools is promising, and we should expect new discoveries in the near future. The use of pressure to study p53C denaturation 10 years ago allowed us to propose that the amyloid aggregation of p53, especially in the case of hot-spot mutants, could have a role in the pathophysiology of malignant tumors. There is also potential to use HHP in the high-throughput screening of compounds that affect the folding-aggregation landscape of proteins involved in neurodegenerative diseases and cancer. As a general conclusion, HHP studies of all of these interactions would yield unique and novel insights into the mechanisms of amyloid formation.

Acknowledgments

The work in the laboratories of J.L.S., Y.C. and D.F. is supported by grants from the Conselho Nacional de Desenvolvimento Cientiíco e Tecnológico (CNPq), Fundacao Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) and Financiadora de Estudos e Projetos (FINEP) of Brazil.

References

[1] J.L. Silva, G. Weber, Pressure stability of proteins, Annual Review of Physical Chemistry 44 (1993) 89–113.
[2] V.V. Mozhaev, K. Heremans, J. Frank, P. Masson, C. Balny, High pressure effects on protein structure and function, Proteins 24 (1996) 81–91.
[3] J.L. Silva, D. Foguel, C.A. Royer, Pressure provides new insights into protein folding, dynamics and structure, Trends in Biochemical Sciences 26 (2001) 612–618.
[4] D. Foguel, J.L. Silva, New insights into the mechanisms of protein misfolding and aggregation in amyloidogenic diseases derived from pressure studies, Biochemistry 43 (2004) 11361–11370.
[5] J. Torrent, S. Marchal, P. Tortora, R. Lange, C. Balny, High pressure, an alternative approach to understand protein misfolding diseases, Cellular and Molecular Biology 50 (2004) 377–385.
[6] P.W. Bridgman, The coagulation of albumen by pressure, Journal of Biological Chemistry 19 (1914) 511–512.
[7] R. Winter, W. Dzwolak, Exploring the temperature–pressure configurational landscape of biomolecules: from lipid membranes to proteins, Philosophical Transactions. Series A, Mathematical, Physical, and Engineering Sciences 363 (2005) 537–562.
[8] K. Akasaka, Probing conformational fluctuation of proteins by pressure perturbation, Chemical Reviews 106 (2006) 1814–1835.
[9] J.L. Silva, Y. Cordeiro, D. Foguel, Protein folding and aggregation: two sides of the same coin in the condensation of proteins revealed by pressure studies, Biochimica et Biophysica Acta 1764 (2006) 443–451.
[10] J.L. Silva, D. Foguel, A.T. Da Poian, P.E. Prevelige, The use of hydrostatic pressure as a tool to study viruses and other macromolecular assemblages, Current Opinion in Structural Biology 6 (1996) 166–175.
[11] J.L. Silva, A.C. Oliveira, A.M. Gomes, L.M. Lima, R. Mohana-Borges, A.B. Pacheco, D. Foguel, Pressure induces folding intermediates that are crucial for protein–DNA recognition and virus assembly, Biochimica et Biophysica Acta 1595 (2002) 250–265.

[12] C.A. Royer, Revisiting volume changes in pressure-induced protein unfolding, Biochimica et Biophysica Acta 1595 (2002) 201–209.
[13] J. Roche, J.A. Caro, D.R. Norberto, P. Barthe, C. Roumestand, J.L. Schlessman, A.E. Garcia, B.E. García-Moreno, C.A. Royer, Cavities determine the pressure unfolding of proteins, Proceedings of the National Academy of Sciences of the United States of America 109 (2012) 6945–6950.
[14] C.B. Anfinsen, The formation and stabilization of protein structure, Biochemical Journal 128 (1972) 737–749.
[15] C.M. Dobson, M. Karplus, The fundamentals of protein folding: bringing together theory and experiments, Current Opinion in Structural Biology 9 (1999) 92–101.
[16] H. Christensen, R.H. Pain, Molten globule intermediates and protein folding, European Biophysics Journal 19 (1991) 221–229.
[17] Z. Lai, W. Colon, J.W. Kelly, The acid-mediated denaturation pathway of transthyretin yields a conformational intermediate which can self-assemble into amyloid, Biochemistry 35 (1996) 6470–6482.
[18] F. Chiti, C.M. Dobson, Protein misfolding, functional amyloid, and human disease, Annual Review of Biochemistry 75 (2006) 333–366.
[19] K.F. Winklhofer, J. Tatzelt, C. Haass, The two faces of protein misfolding: gain- and loss-of-function in neurodegenerative diseases, EMBO Journal 27 (2008) 336–349.
[20] F.U. Hartl, M. Hayer-Hartl, Molecular chaperones in the cytosol: from nascent chain to folded protein, Science 295 (2002) 1852–1858.
[21] C.J. Guerriero, J.L. Brodsky, The delicate balance between secreted protein folding and endoplasmic reticulum-associated degradation in human physiology, Physiological Reviews 92 (2012) 537–576.
[22] A. Horwich, Protein aggregation in disease: a role for folding intermediates forming specific multimeric interactions, The Journal of Clinical Investigation 110 (2002) 1221–1232.
[23] B. Caughey, P.T. Lansbury, Protofibrils, pores, fibrils, and neurodegeneration: separating the responsible protein aggregates from the innocent bystanders, Annual Review of Neuroscience 26 (2003) 267–298.
[24] M. Sunde, C.C. Blake, The structure of amyloid fibrils by electron microscopy and X-ray diffraction, Advances in Protein Chemistry 50 (1997) 123–159.
[25] C. Ritter, M.L. Maddelein, A.B. Siemer, T. Luhrs, M. Ernst, B.H. Meier, S.J. Saupe, R. Riek, Correlation of structural elements and infectivity of the HET-s prion, Nature 435 (2005) 844–848.
[26] J. Ries, V. Udayar, A. Soragni, S. Hornemann, K.P. Nilsson, R. Riek, C. Hock, H. Ewers, A. Aguzzi, L. Rajendran, Superresolution imaging of amyloid fibrils with binding-activated probes, ACS Chemical Neuroscience (2013), (accepted manuscript).
[27] A.W. Fitzpatrick, G.T. Debelouchina, M.J. Bayro, D.K. Clare, M.A. Caporini, V.S. Bajaj, C.P. Jaroniec, L. Wang, V. Ladizhansky, S.A. Müller, C.E. Macphee, C.A. Waudby, H.R. Mott, A. De Simone, T.P. Knowles, H.R. Saibil, M. Vendruscolo, E.V. Orlova, R.G. Griffin, C.M. Dobson, Atomic structure and hierarchical assembly of a cross-β amyloid fibril, Proceedings of the National Academy of Sciences of the United States of America 110 (2013) 5468–5473.
[28] C.M. Dobson, Protein misfolding, evolution and disease, Trends in Biochemical Sciences 24 (1999) 329–332.
[29] C.M. Dobson, Principles of protein folding, misfolding and aggregation, Seminars in Cell & Developmental Biology 15 (2004) 3–16.
[30] J.W. Kelly, The environmental dependency of protein folding best explains prion and amyloid disease, Proceedings of the National Academy of Sciences of the United States of America 95 (1998) 930–932.
[31] A.D. Ferrão-Gonzales, S.O. Souto, J.L. Silva, D. Foguel, The preaggregated state of an amyloidogenic protein: hydrostatic pressure converts native transthyretin into the amyloidogenic state, Proceedings of the National Academy of Sciences of the United States of America 97 (2000) 6445–6450.
[32] A.D. Ferrão-Gonzales, L. Palmieri, M. Valory, J.L. Silva, H. Lashuel, J.W. Kelly, D. Foguel, Hydration and packing are crucial to amyloidogenesis as revealed by pressure studies on transthyretin variants that either protect or worsen amyloid disease, Journal of Molecular Biology 328 (2003) 963–974.
[33] D. Foguel, M.C. Suarez, A.D. Ferrão-Gonzales, T.C. Porto, L. Palmieri, C.M. Einsiedler, L.R. Andrade, H.A. Lashuel, P.T. Lansbury, J.W. Kelly, J.L. Silva, Dissociation of amyloid fibrils of alpha-synuclein and transthyretin by pressure reveals their reversible nature and the formation of water-excluded cavities, Proceedings of the National Academy of Sciences of the United States of America 100 (2003) 9831–9836.
[34] T.N. Niraula, T. Konno, H. Li, H. Yamada, K. Akasaka, H. Tachibana, Pressure-dissociable reversible assembly of intrinsically denatured lysozyme is a precursor for amyloid fibrils, Proceedings of the National Academy of Sciences of the United States of America 101 (2004) 4089–4093.
[35] Y. Cordeiro, J. Kraineva, R. Ravindra, L.M.T.R. Lima, M.P. Gomes, D. Foguel, R. Winter, J.L. Silva, Hydration and packing effects on prion folding and beta-sheet conversion. High pressure spectroscopy and pressure perturbation calorimetry studies, Journal of Biological Chemistry 279 (2004) 32354–32359.
[36] C. Follmer, L. Romão, C.M. Einsiedler, T.C. Porto, F.A. Lara, M. Moncores, G. Weissmüller, H.A. Lashuel, P. Lansbury, V.M. Neto, J.L. Silva, D. Foguel, Dopamine affects the stability, hydration, and packing of protofibrils and fibrils of the wild type and variants of alpha-synuclein, Biochemistry 46 (2007) 472–482.
[37] D. El Moustaine, V. Perrier, I. Acquatella-Tran Van Ba, F. Meersman, V.G. Ostapchenko, I.V. Baskakov, R. Lange, J. Torrent, Amyloid features and neuronal toxicity of mature prion fibrils are highly sensitive to high pressure, Journal of Biological Chemistry 286 (2011) 13448–13459.
[38] S.B. Prusiner, Novel proteinaceous infectious particles cause scrapie, Science 216 (1982) 136–144.
[39] A. Aguzzi, M. Polymenidou, Mammalian prion biology: one century of evolving concepts, Cell 116 (2004) 313–327.
[40] Y. Cordeiro, F. Machado, L. Juliano, M.A. Juliano, R.R. Brentani, D. Foguel, J.L. Silva, DNA converts cellular prion protein into the beta-sheet conformation and inhibits prion peptide aggregation, Journal of Biological Chemistry 276 (2001) 49400–49409.
[41] N.R. Deleault, B.T. Harris, J.R. Rees, S. Supattapone, Formation of native prions from minimal components in vitro, Proceedings of the National Academy of Sciences of the United States of America 104 (2007) 9741–9746.
[42] J.L. Silva, M.P.B. Gomes, T.C.G. Vieira, Y. Cordeiro, PrP interactions with nucleic acids and glycosaminoglycans in function and disease, Frontiers in Bioscience 15 (2010) 132–150.
[43] S.B. Prusiner, Prions, Proceedings of the National Academy of Sciences of the United States of America 95 (1998) 13363–13383.
[44] R. Zahn, A. Liu, T. Lührs, R. Riek, C. von Schroetter, F. López García, M. Billeter, L. Calzolai, G. Wider, K. Wüthrich, NMR solution structure of the human prion protein, Proceedings of the National Academy of Sciences of the United States of America 97 (2000) 145–150.
[45] F. Wopfner, G. Weidenhöfer, R. Schneider, A. von Brunn, S. Gilch, T.F. Schwarz, T. Werner, H.M. Schätzl, Analysis of 27 mammalian and 9 avian PrPs reveals high conservation of flexible regions of the prion protein, Journal of Molecular Biology 289 (1999) 1163–1178.
[46] M.P. Gomes, T.C. Vieira, Y. Cordeiro, J.L. Silva, The role of RNA in mammalian prion protein conversion, WIREs RNA 3 (2012) 415–428.
[47] Z. Huang, S.B. Prusiner, F.E. Cohen, Scrapie prions: a three-dimensional model of an infectious fragment, Folding and Design 1 (1996) 13–19.
[48] C. Govaerts, H. Wille, S.B. Prusiner, F.E. Cohen, Evidence for assembly of prions with left-handed beta-helices into trimers, Proceedings of the National Academy of Sciences of the United States of America 101 (2004) 8342–8347.
[49] B. Gong, A. Ramos, E. Vázquez-Fernández, C.J. Silva, J. Alonso, Z. Liu, J.R. Requena, Probing structural differences between PrP(C) and PrP(Sc) by surface nitration and acetylation: evidence of conformational change in the C-terminus, Biochemistry 50 (2011) 4963–4972.
[50] J. Torrent, M.T. Alvarez-Martinez, F. Heitz, J.P. Liautard, C. Balny, R. Lange, Alternative prion structural changes revealed by high pressure, Biochemistry 42 (2003) 1318–1325.
[51] Y.O. Kamatari, S. Yokoyama, H. Tachibana, K. Akasaka, Pressure-jump NMR study of dissociation and association of amyloid protofibrils, Journal of Molecular Biology 349 (2005) 916–921.
[52] Y. Cordeiro, L.M.T.R. Lima, M.P.B. Gomes, D. Foguel, J.L. Silva, Modulation of prion protein oligomerization, aggregation, and beta-sheet conversion by 4,4′-dianilino-1,1′-binaphthyl-5,5′-sulfonate (bis-ANS), Journal of Biological Chemistry 279 (2004) 5346–5352.
[53] J. Torrent, M.T. Alvarez-Martinez, M.C. Harricane, F. Heitz, J.P. Liautard, C. Balny, R. Lange, High pressure induces scrapie-like prion protein misfolding and amyloid fibril formation, Biochemistry 43 (2004) 7162–7170.
[54] D. El Moustaine, V. Perrier, L. Smeller, R. Lange, J. Torrent, Full-length prion protein aggregates to amyloid fibrils and spherical particles by distinct pathways, FEBS Journal 275 (2008) 2021–2031.
[55] Y. Cordeiro, J. Kraineva, M.P. Gomes, M.H. Lopes, V.R. Martins, L.M.T.R. Lima, D. Foguel, R. Winter, J.L. Silva, The amino-terminal PrP domain is crucial to modulate prion misfolding and aggregation, Biophysical Journal 89 (2005) 2667–2676.
[56] N. Kachel, W. Kremer, R. Zahn, H.R. Kalbitzer, Observation of intermediate states of the human prion protein by high pressure NMR spectroscopy, BMC Structural Biology 6 (2006) 16.
[57] W. Kremer, N. Kachel, K. Kuwata, K. Akasaka, H.R. Kalbitzer, Species-specific differences in the intermediate states of human and Syrian hamster prion protein detected by high pressure NMR spectroscopy, Journal of Biological Chemistry 282 (2007) 22689–22698.
[58] K. Kuwata, H. Li, H. Yamada, G. Legname, S.B. Prusiner, K. Akasaka, T.L. James, Locally disordered conformer of the hamster prion protein: a crucial intermediate to PrPSc? Biochemistry 41 (2002) 12277–12283.
[59] J. Torrent, R. Lange, High pressure, a tool to switch between soluble and fibrillar prion protein structures, Communicative & Integrative Biology 5 (2012) 30–33.
[60] A.F. Garcia, P. Heindl, H. Voigt, M. Büttner, P. Butz, N. Tauber, B. Tauscher, E. Pfaff, Dual nature of the infectious prion protein revealed by high pressure, Journal of Biological Chemistry 280 (2005) 9842–9847.
[61] F. Cardone, P. Brown, R. Meyer, M. Pocchiari, Inactivation of transmissible spongiform encephalopathy agents in food products by ultra high pressure–temperature treatment, Biochimica et Biophysica Acta 1764 (2006) 558–562.
[62] K.H. Vousden, D.P. Lane, p53 in health and disease, Nature Reviews Molecular Cell Biology 8 (2007) 275–283.
[63] P.A. Muller, K.H. Vousden, p53 mutations in cancer, Nature Cell Biology 15 (2013) 2–8.
[64] A.C. Joerger, A.R. Fersht, Structural biology of the tumor suppressor p53, Annual Review of Biochemistry 77 (2008) 557–582.
[65] A.N. Bullock, J. Henckel, B.S. DeDecker, C.M. Johnson, P.V. Nikolova, M.R. Proctor, D.P. Lane, A.R. Fersht, Thermodynamic stability of wild-type and mutant p53 core domain, Proceedings of the National Academy of Sciences of the United States of America 94 (1997) 14338–14342.
[66] D. Ishimaru, L.R. Andrade, L.S. Teixeira, P.A. Quesado, L.M. Maiolino, P.M. Lopez, Y. Cordeiro, L.T. Costa, W.M. Heckl, G. Weissmüller, D. Foguel, J.L. Silva, Fibrillar aggregates of the tumor suppressor p53 core domain, Biochemistry 42 (2003) 9022–9027.
[67] D. Ishimaru, A.P. Ano Bom, L.M. Lima, P.A. Quesado, M.F. Oyama, C.V. de Moura Gallo, Y. Cordeiro, J.L. Silva, Cognate DNA stabilizes the tumor suppressor p53 and prevents misfolding and aggregation, Biochemistry 48 (2009) 6126–6135.

Please cite this article as: Y. Cordeiro, et al., Pressure–temperature folding landscape in proteins involved in neurodegenerative diseases and cancer, Biophysical Chemistry (2013), http://dx.doi.org/10.1016/j.bpc.2013.06.002

[68] J.L. Silva, T.C. Vieira, M.P. Gomes, A.P. Ano Bom, L.M. Lima, M.S. Freitas, D. Ishimaru, Y. Cordeiro, D. Foguel, Ligand binding and hydration in protein misfolding: insights from studies of prion and p53 tumor suppressor proteins, Accounts of Chemical Research 43 (2010) 271–279.
[69] C.B. Levy, A.C. Stumbo, A.P. Ano Bom, E.A. Portari, Y. Cordeiro, J.L. Silva, C.V. De Moura-Gallo, Co-localization of mutant p53 and amyloid-like protein aggregates in breast tumors, The International Journal of Biochemistry & Cell Biology 43 (2011) 60–64.
[70] J. Xu, J. Reumers, J.R. Couceiro, F. De Smet, R. Gallardo, S. Rudyak, A. Cornelis, J. Rozenski, A. Zwolinska, J.C. Marine, D. Lambrechts, Y.A. Suh, F. Rousseau, J. Schymkowitz, Gain of function of mutant p53 by coaggregation with multiple tumor suppressors, Nature Chemical Biology 7 (2011) 285–295.
[71] A.P. Ano Bom, L.P. Rangel, D.C. Costa, G.A. de Oliveira, D. Sanches, C.A.C.A. Braga, L.M. Gava, C.H. Ramos, A.O. Cepeda, A.C. Stumbo, C.V. De Moura Gallo, Y. Cordeiro, J.L. Silva, Mutant p53 aggregates into prion-like amyloid oligomers and fibrils: implications for cancer, Journal of Biological Chemistry 287 (2012) 28152–28162.
[72] R. Wilcken, G. Wang, F.M. Boeckler, A.R. Fersht, Kinetic mechanism of p53 oncogenic mutant aggregation and its inhibition, Proceedings of the National Academy of Sciences of the United States of America 109 (2012) 13584–13589.
[73] C.A. Lasagna-Reeves, A.L. Clos, D. Castillo-Carranza, U. Sengupta, M. Guerrero-Muñoz, B. Kelly, R. Wagner, R. Kayed, Dual role of p53 amyloid formation in cancer; loss of function and gain of toxicity, Biochemical and Biophysical Research Communications 430 (2013) 963–968.
[74] D. Ishimaru, L.F. Maia, L.M. Maiolino, P.A. Quesado, P.C. Lopez, F.C. Almeida, A.P. Valente, J.L. Silva, Conversion of wild-type p53 core domain into a conformation that mimics a hot-spot mutant, Journal of Molecular Biology 333 (2003) 443–451.
[75] K.S. Vassilenko, V.N. Uversky, Native-like secondary structure of molten globules, Biochimica et Biophysica Acta 1594 (2002) 168–177.
[76] A.P. Ano Bom, M.S. Freitas, F.S. Moreira, D. Ferraz, D. Sanches, A.M. Gomes, A.P. Valente, Y. Cordeiro, J.L. Silva, The p53 core domain is a molten globule at low pH: functional implications of a partially unfolded structure, Journal of Biological Chemistry 285 (2010) 2857–2866.
[77] L. Xu, D. Fukumura, R.K. Jain, Acidic extracellular pH induces vascular endothelial growth factor (VEGF) in human glioblastoma cells via ERK1/2 MAPK signaling pathway: mechanism of low pH-induced VEGF, Journal of Biological Chemistry 277 (2002) 11368–11374.
[78] C.C. Blake, M.J. Geisow, S.J. Oatley, B. Rerat, C. Rerat, Structure of prealbumin: secondary, tertiary and quaternary interactions determined by Fourier refinement at 1.8 Å, Journal of Molecular Biology 121 (1978) 339–356.
[79] J.N. Buxbaum, N. Reixach, Transthyretin: the servant of many masters, Cellular and Molecular Life Sciences 66 (2009) 3095–30101.
[80] P. Westermark, K. Sletten, B. Johansson, G.G. Cornwell, Fibril in senile systemic amyloidosis is derived from normal transthyretin, Proceedings of the National Academy of Sciences of the United States of America 87 (1990) 2843–2845.
[81] M.J. Saraiva, Transthyretin mutations in hyperthyroxinemia and amyloid diseases, Human Mutation 17 (2001) 493–503.
[82] A.T. Da Poian, A.C. Oliveira, J.L. Silva, Cold denaturation of an icosahedral virus. The role of entropy in virus assembly, Biochemistry 34 (1995) 2672–2677.
[83] R.O. Sant'anna, C.A. Braga, I. Polikarpov, S. Ventura, L.M. Lima, D. Foguel, Inhibition of human transthyretin aggregation by non-steroidal anti-inflammatory compounds: a structural and thermodynamic analysis, International Journal of Molecular Sciences 14 (2013) 5284–52311.
[84] F.L. Palhano, L.P. Leme, R.G. Busnardo, D. Foguel, Trapping the monomer of a non-amyloidogenic variant of transthyretin: exploring its possible use as a therapeutic strategy against transthyretin amyloidogenic diseases, Journal of Biological Chemistry 284 (2009) 1443–1453.
[85] L.S. Forno, Neuropathology of Parkinson's disease, Journal of Neuropathology and Experimental Neurology 55 (1996) 259–272.
[86] M.G. Spillantini, M.L. Schmidt, V.M. Lee, J.Q. Trojanowski, R. Jakes, M. Goldberg, a-Synuclein in Lewy bodies, Nature 388 (1997) 839–840.
[87] M. Baba, S. Nakajo, P.H. Tu, T. Tomita, K. Nakaya, V.M. Lee, J.Q. Trojanowsky, T. Iwatsubo, Aggregation of a-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies, American Journal of Pathology 152 (1998) 879–884.
[88] S.B. Dunnet, A. Bjorklund, Prospect for new restorative and neuroprotective treatments in Parkinson's disease, Nature 399 (1999) A32–A39.
[89] K.A. Conway, S.-J. Lee, J.-C. Rochet, T.T. Ding, R.E. Williamson, P.T. Lansbury, Acceleration of oligomerization, not fibrillization, is a shared property of both α-synuclein mutations linked to early-onset Parkinson's disease: implication for pathogenesis and therapy, Proceedings of the National Academy of Sciences of the United States of America 97 (2000) 571–576.
[90] M.H. Polymeropoulos, C. Lavendan, E. Leroy, S.E. Ide, A. Dehejia, A. Dutra, B. Pike, H. Root, J. Rubenstein, R. Boyer, Mutation in the alpha-synuclein gene identified in families with Parkinson's disease, Science 276 (1997) 2045–2047.
[91] K.A. Conway, J.D. Harper, P.T. Lansbury, Accelerated in vitro fibril formation by a mutant alpha-synuclein linked to early-onset Parkinson disease, Nature Medicine 4 (1998) 1318–1320.
[92] C. Dirix, F. Meersman, C.E. MacPhee, C.M. Dobson, K. Heremans, High hydrostatic pressure dissociates early aggregates of TTR105–115, but not the mature amyloid fibrils, Journal of Molecular Biology 347 (2005) 903–909.
[93] E. Chatani, Y. Goto, Structural stability of amyloid fibrils of beta(2)-microglobulin in comparison with its native fold, Biochimica et Biophysica Acta 1753 (2005) 64–75.
[94] D. Radovan, V. Smirnovas, R. Winter, Effect of pressure on islet amyloid polypeptide aggregation: revealing the polymorphic nature of the fibrillation process, Biochemistry 47 (2008) 6352–6360.
[95] S.T. Ferreira, A. Chapeaurouge, F.G. De Felice, Stabilization of partially folded states in protein folding/misfolding transitions by hydrostatic pressure, Brazilian Journal of Medical and Biological Research 38 (2005) 1215–1222.
[96] H.A. Lashuel, D. Hartley, B.M. Petre, T. Walz, P.T. Lansbury Jr., Neurodegenerative disease: amyloid pores from pathogenic mutations, Nature 418 (2002) 291.
[97] M. Stefani, C.M. Dobson, Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution, Journal of Molecular Medicine 81 (2003) 678–699.
[98] M. Bucciantini, E. Giannoni, F. Chiti, F. Baroni, L. Formigli, J. Zurdo, N. Taddei, G. Ramponi, C.M. Dobson, M. Stefani, Inherent cytotoxicity of aggregates implies a common origin for protein misfolding diseases, Nature 416 (2002) 507–511.

Please cite this article as: Y. Cordeiro, et al., Pressure–temperature folding landscape in proteins involved in neurodegenerative diseases and cancer, Biophysical Chemistry (2013), http://dx.doi.org/10.1016/j.bpc.2013.06.002