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Review

Tuning amyloidogenic conformations through cosolvents and hydrostatic pressure: When the soft matter becomes even softer

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Abstract

Compact packing, burial of hydrophobic side-chains, and low free energy levels of folded conformations contribute to stability of native proteins. Essentially, the same factors are implicated in an even higher stability of mature amyloid fibrils. Although both native insulin and insulin amyloid are resistant to high pressure and influence of cosolvents, intermediate aggregation-prone conformations are susceptible to either condition. Consequently, insulin fibrillation may be tuned under hydrostatic pressure or – through cosolvents and cosolutes – by preferential exclusion or binding. Paradoxically, under high pressure, which generally disfavors aggregation of insulin, an alternative "low-volume" aggregation pathway, which leads to unique circular amyloid is permitted. Likewise, cosolvents are capable of preventing, or altering amyloidogenesis of insulin. As a result of cosolvent-induced perturbation, distinct conformational variants of fibrils are formed. Such variants, when used as templates for seeding daughter generations, reproduce initial folding patterns regardless of environmental biases. By the close analogy, this suggests that the "prion strains" phenomenon may mirror a generic, common feature in amyloids. The susceptibility of amyloidogenic conformations to pressure and cosolvents is likely to arise from their "frustration", as unfolding results in less-densely packed side-chains, void volumes, and exposure of hydrophobic groups. The effects of cosolvents and pressure are discussed in the context of studies on other amyloidogenic protein models, amyloid polymorphism, and "strains".

Keywords: Protein aggregation; Insulin; Amyloid; High pressure; Cosolvent; Preferential hydration; Seeding

1. Introduction

Amyloids, the non-native β-sheet-rich protein fibrils, were implicated in more than 20 human disorders, such as Alzheimer Disease, Parkinson Disease, or the "prion"-associated Creutzfeldt–Jakob Disease [1–3]. Since then protein aggregation has become one of the most thoroughly studied topics in molecular biology. The hypothesis pinpointing origins of such conditions to amyloid deposits has recently been challenged by studies showing lack of correlation between the amount of amyloidal deposit in tissue and the degree of histological damage. It appears that certain fibrillation-prone, partly unstructured, intermediate states – but not the amyloid fibrils per se – may be neuro- or in general: cytotoxic [4,5]. For example, early oligomeric protofibrils of α-synuclein rather than mature fibrils appear to be the actual culprit of neurotoxicity in Parkinson Disease [6]. In light of these studies, fibrillogenesis may be deemed a "rescue" tactic, which limits amount of the most detrimental conformational form of a misfolded protein, and – as a consequence – the degree of histological damage. All in all, however, protein aggregation appears to pose a threat rather than opportunity to life on its molecular, cellular and histological levels. There are few convincing examples when an organism is benefiting from amyloidogenesis of its own proteins: the best characterized being "yeast prions", such as [URE3], or [PSI^+] which constitute a pattern of inheritance of certain metabolic regulations in yeasts

Abbreviations: AFM, Atomic Force Microscopy; ASA, Accessible Surface Area; CD, Circular Dichroism; DAC, Diamond Anvil Cell; DSC, Differential Scanning Calorimetry; EtOH, Ethanol; FT-IR, Fourier Transform Infrared Spectroscopy; Ig-LC, Immunoglobulin Light Chain; NMR, Nuclear Magnetic Resonance; PPC, Pressure Perturbation Calorimetry; PrP^C, Native Cellular Isoform of Prion Protein; PrP^{Sc}, Scrapie Isoform of Prion Protein; TTR, Transthyretin; [URE3], [PSI^+], 'yeast prions'—heritable traits in *Saccharomyces cerevisiae* formed by cellular proteins: Ure2p and Sup35p, respectively

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[7,8]. Arguably, another possible case concerns Pmel17 protein involved in biogenesis of melanosomes in humans [9]. An idea that amyloid-type proteinaceous self-organization played a role in proliferation of primordial structures has been explored elsewhere [10]. Nowadays, the protein aggregation also posses a major problem in biotechnology when so-called inclusion bodies are formed and efficiency of overexpressed recombinant proteins is spoilt. While the rather narrow medical context remains the main (and unrelenting) rationale for undertaking studies in this field [1–3], from a physicochemical perspective, protein aggregation in vivo may represent a limited aspect of a common and generic among proteins (as polymers) feature [11]. In Table 1, a number of classical amyloidogenic proteins are juxtaposed with "benign" proteins, short synthetic peptides, or even sequenceless polymerized amino acids capable of forming in vitro fibrils with an amyloid-like outlook. A brief survey of the data suggests that:

- Formation of fibrillar β-sheet-rich aggregates is a feature shared by genetically unrelated (e.g. PrP^C, insulin, and cytochrome), or even sequenceless (polylysine) proteins and polypeptides,
- The simplest systems, such as the KFFE peptide, hint at aggregation being possibly driven by periodic charge-compensation, and clustering of hydrophobic residues, which – by a motive repetition – explain the self-assembling into infinitive structures,
- Aggregation of short and presumably orderless peptides occurs under ambient conditions, whereas folded proteins require prolonged incubation under destabilizing conditions such as high temperature, high or low pH, intensive shaking, high-pressure treatment, freeze–thawing cycles, sonification, but perhaps most often: presence of structure-disrupting cosolvents, denaturants, or mutations.

Thus, a disordered conformation is either the starting point for amyloidogenesis (as is the case of natively unfolded proteins [32] and peptides [14–18]) or a transient state that must occur on native proteins' amyloidogenic pathways (Fig. 1). Residual, unordered conformations are also likely to be one of the critical

Table 1
Examples of amyloidogenic peptides and proteins

Peptide/protein	Conditions of fibrillation	Properties of the aggregate/fibrils	Remarks	Reference
Poly(L-lysine), poly(L-threonine), poly(L-glutamic acid)	65 °C and charge depleting pH (alkalic for K, acidic for E)	From straight, unbranched to short and twisted	Main chain interactions promote aggregation	[11]
Poly(L-lysine)+poly(D-lysine),	Mixing at room temp. and alkalic pH	Highly distorted	Racemic fibrils form due to increase in water ΔS	[12]
FF [a]	Precipitation upon dilution with water	Regular "empty" nanotubes	Form "nanowires" when filled with Ag atoms	[13]
KVVE, KFFE [a]	Long incubation at 37 °C, neutral pH	Bundles resembling in vivo deposits	Charges compensated in the antiparallel alignment	[14]
$(DPKG)_2$-$(VT)_{3..8}$-GKGDPKPD-NH_2 [a]	Spontaneous fibrillation at 25 °C	Sequence-dependent morphologies	Monomers and early fibrils are soluble	[15]
Alzheimer β-peptide fragments	Aggregation dependent on pH	CD spectra same as for in vivo deposits	The pH-range parallels in vivo conditions	[16–18]
Oxidized glutathione (GSSG) [a]	Aqueous solutions of DMSO, DMF, MeOH	Thick, 75 nm in diameter fibrils	Network with β-sheet like motive traps solvent	[19]
Cyclic octa-peptides alternating D/L amino acids	Precipitation at acidic pH in the presence of cosolvent	Fibrils 1.5 nm thick with an internal diameter 0.7–0.8 nm	Open-ended "nanotubes" with a regular β-sheet like motive	[20,21]
Bovine serum albumin	Sonification at room temperature	Heterogeneous fibrils, 20 nm thick	Possible role of free radicals in the aggregation	[22]
Antifreeze protein of winter flounder	Repetitive freezing–thawing cycles.	Short, 5 nm in diameter	Ice-binding may trigger the fibrillation	[23]
C11A/C14A mut. cytochrome c_{552}	Incubation at 25 °C, neutral pH	Typical fibrils are 6–13 nm thick	C11A/C14A substitution destabilizes *holo*-protein	[24]
Horse *apo*-myoglobin	A day-long incubation at 65 °C, pH 9	Fibrils are unstable above 65 °C	Amyloidogenesis requires complete unfolding	[25]
Bovine insulin	Hours-long incubation at 65 °C, pH 2	Fibrils associated in spherulites	Characteristic optical properties	[26]
Tetramerization domain p53 protein	Gradual heating up to 95 °C, pH 4	Straight and curly forms 1–2 nm thick	Amyloid disassembles above pH 8.5	[27]
Transthyretin	3.5 kbar pressure, at 37 °C, neutral pH	Formed after depressurization	Non-native aggregation-prone tetramers formed	[28]
Hen egg white lysozyme	2–5 M guanidine HCl, 50 °C, neutral pH	Twisted, approx. 13 nm thick	Denaturant concentration within range of 2 and 5 M	[29]
Yeast prion protein Ure2	Shaking at 37 °C, pH 8	Straight fibrils, 4–8 nm in height (AFM)	Highly heterogeneous samples	[30]
Prion proteins: Human PrP(90-231), Mouse PrP (89-230)	Shaking at 37 °C, at pH ranging from 3.7 to 7.2	Fibrils at neutral pH shorter than those formed at pH 3.7	Fibrillation enhanced by homologous seeding (Hu→Hu, Mo→Mo)	[31]

[a] Peptide names decode amino acid sequence according to the one-letter system.