Chemical catalysis by biological amyloids

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Review Article

Chemical catalysis by biological amyloids

Pernilla Wittung-Stafshede

Department of Life Sciences, Chalmers University of Technology, 412 96 Gothenburg, Sweden

Correspondence: Pernilla Wittung-Stafshede (pernilla.wittung@chalmers.se)

Toxic aggregation of proteins and peptides into amyloid fibers is the basis of several human diseases. In each disease, a particular peptide noncovalently assembles into long thin structures with an overall cross-β fold. Amyloids are not only related to disease: functional amyloids are found in many biological systems and artificial peptide amyloids are developed into novel nanomaterials. Amyloid fibers can act as template for the generation of more amyloids but are considered nonreactive in chemical catalysis. The perception of amyloids as chemically inert species was recently challenged by *in vitro* work on three human amyloid systems. With the use of model substrates, amyloid-β, α-synuclein and glucagon amyloids were found to catalyze biologically relevant chemical reactions. The detected catalytic activity was much less than that of 'real' enzymes, but like that of designed (synthetic) catalytic amyloids. I here describe the current knowledge around this new activity of natural amyloids and the putative connection to metabolic changes in amyloid diseases. These pioneering studies imply that catalytic activity is an unexplored gain-of-function activity of disease amyloids. In fact, all biological amyloids may harbor intrinsic catalytic activity, tuned by each amyloid's particular fold, that await discovery.

Amyloids in disease ('bad' amyloids)

Amyloid fibrils are polymers of monomeric protein units noncovalently assembled through β-strands arranged perpendicularly to the long axis, forming a cross-β structure [1]. Many proteins can form amyloid fibrils at certain solvent conditions *in vitro* [1], but this process is mostly connected to human neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases [2]. In addition to brain diseases, type-2 diabetes involves amyloid formation in the pancreas, and systemic amyloidosis disorders involve amyloid deposition in many different organs [3,4]. In fact, over 50 different proteins or peptides are known today to assemble into pathological amyloids [5]. Although each protein has a different primary sequence, they are unified by the common amyloid scaffold: elongated, unbranched structures (5–20 nm wide, up to micrometers long) of protein monomers stacked on top of each other in β-strand motifs stabilized by steric hydrophobic 'zippers' [6].

In most amyloid diseases, the amyloid fibrils are considered end products that merely accumulate in extracellular plaque or as intracellular inclusions. Instead, intermediate species (so-called oligomers or pre-fibrillar species) formed during the assembly process towards amyloids are thought to be most toxic [7–9]. Still, the amyloid fibrils themselves are 'bad' too: they can transfer from cell to cell, spreading the disease, and act as templates for the generation of new amyloids via elongation and secondary processes [10–13]. Deleterious biological consequences coupled to the formation of amyloid fibers include, for example, mitochondrial dysfunction, oxidative stress, metal dys-homeostasis, protein degradation failure, and eventually cell death [7,14–16].

Despite decades of research on amyloid diseases and amyloid-forming proteins, we only have some disease-modifying therapies in use [17–19]. The exception is Tafamidis, a small molecule that stops transthyretin-related amyloidosis by stabilizing the folded, tetrameric form of the amyloidogenic protein transthyretin [20,21]. Reasons for the lack of cures are, among others, that many amyloidogenic proteins are intrinsically disordered (and thus monomers are hard to target), most of these diseases build up over multiple decades (when clinical manifestation is observed, it is too late), and the

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diseases are often multifactorial and depend on a combination of genetics, environmental factors, and age. Clearly, more fundamental research on triggers, mechanisms, and consequences of amyloids are needed.

Functional amyloids ('good' amyloids)

Amyloid formation is not only connected to human disease. There is a large number of proteins assembling into functional amyloids in all kingdoms of life [22]. For example, functional amyloids stabilize bacterial biofilms, provide scaffolding for melanin synthesis, store peptide hormones, and facilitate interactions between proteins in subcellular condensates [6,23]. Still, non-human functional amyloids may contribute to human disease, for example by enabling bacterial pathogenicity [24] and by seeding of aggregation of human amyloidogenic proteins [25].

The amyloid scaffold has also been used as a template for the development of functional nanomaterials. Short synthetic peptides have been designed to form amyloid structures that assemble as either monolayers at interfaces or as elongated amyloid bilayers. Such synthetic amyloid structures may be used as biocompatible materials for drug delivery, antimicrobial agents, biosensor devices, molecular electronics, and for catalytic reactions [26]. Synthetic amyloids strategically designed to display catalytic sites (protruding catalytic domains or engineered metal-binding sites) on the amyloid surface have been shown to catalyze reactions such as ester hydrolysis, oxidative polymerization, aldol-condensation, peroxidation, and others [27,28]. Synthetic peptides as short as 3–7 residues can assemble into amyloids with catalytic activities [28,29]. In these types of peptide designs, catalytic activity is thought to result from strategic positioning of hydrophilic residues (e.g. His, Ser, Glu, Lys; combinations of these) on the amyloid surface that creates substrate-binding sites.

All published studies of catalytic amyloids have used tailor-made *de novo*-designed peptide sequences. It was not considered that natural amyloidogenic peptides or proteins would harbor intrinsic catalytic activity. Recent work now challenges the concept of natural amyloids as chemically inert species. Below, I describe what is known so far about this emerging amyloid activity, its implications for human disease, and desired future explorations.

New function of physiological amyloids?

In publications from 2021 and 2022, respectively, Jelenik et al. [30] reported that amyloid fibers (but not the monomers) of the Alzheimer's disease peptide amyloid-β (Aβ) and of the glucose-regulating hormone glucagon [31] catalyzed pathological and metabolic chemical transformations *in vitro*. In 2023, my group reported similar *in vitro* activity for amyloid fibers of the amyloidogenic protein in Parkinson's disease, α-synuclein [32]. We recently extended our investigation to putative catalytic activity of α-synuclein amyloids on real metabolites from neuronal cells [33].

In Figure 1, reported high-resolution structures of amyloid-β, α-synuclein, and glucagon amyloids are shown [6]. I note that assembly of a particular polypeptide into an amyloid can result in many different amyloid polymorphs (depending on conditions, but also on unknown factors). The structures shown here are for amyloids generated *in vitro* of the selected proteins (to relate to the studies presented below) and should be considered merely examples of possible amyloid folds of that protein/peptide.

Chemical catalysis by amyloid-β amyloids

To test if a pathological amyloid can promote chemical transformations, several reactions were investigated *in vitro* in the presence of amyloid-β(1−42) amyloids [30]. Esterase activity was tested using the common model substrate para-nitrophenyl acetate (pNPA). Amyloids, but not monomeric peptides, readily catalyzed pNPA hydrolysis (Table 1). Addition of metal ions did not improve the activity. To assess the role of different segments of the 42-residue amyloid-β peptide, shorter variants were tested for activity. Although amyloid-β(1–22) and amyloid-β(16–22) peptides formed amyloids, they did not exhibit esterase activity. Next, chemical reactions directly associated with neurodegeneration were probed. Amyloid-β amyloids were found to catalyze hydrolysis of acetylthiocholine (an acetylcholine surrogate; disruption of acetylcholine signaling is a hallmark of neurodegeneration) as well as oxidation of dopamine and adrenaline (neurotransmitters; dopamine oxidation has been reported in Alzheimer's disease) ([30]). The authors proposed that synergy between hydrophobicity (substrate binding) and nucleophilic side chains (reactivity) on the amyloid fibers promoted the multi-step process of catecholamine oxidation.

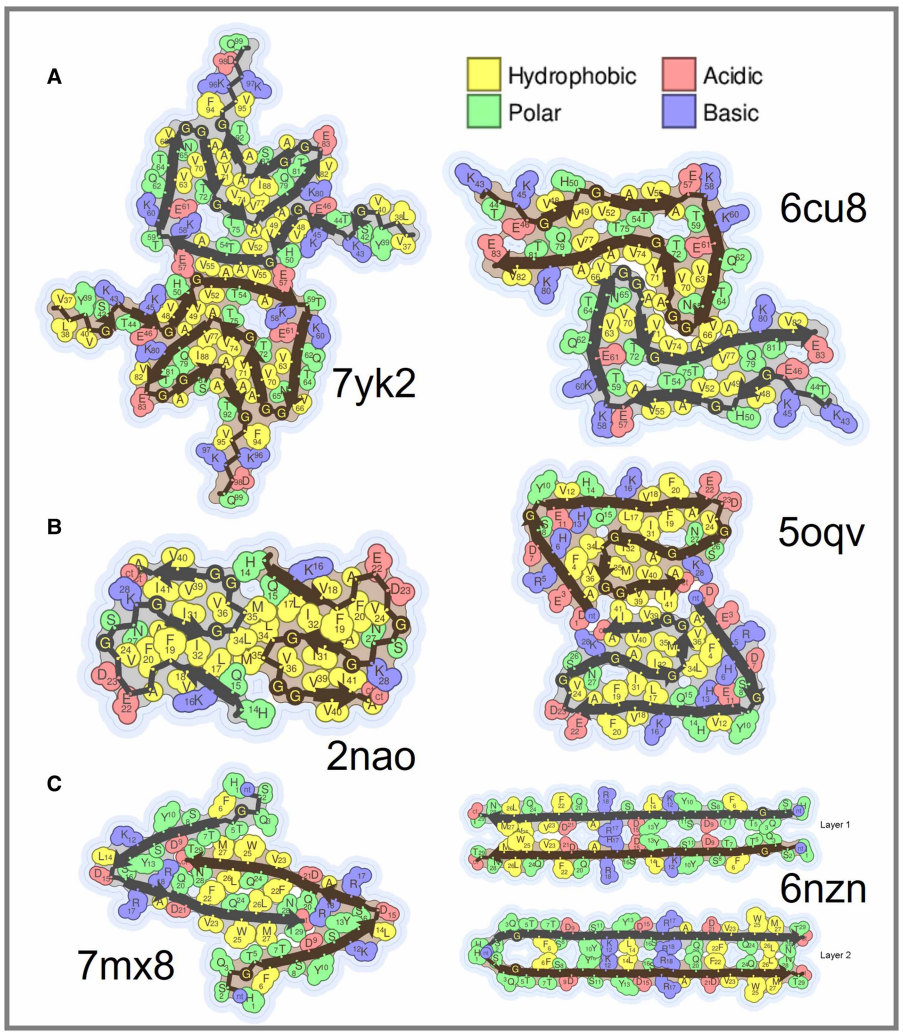

Figure 1. High-resolution structures of amyloid folds adopted by α-synuclein, amyloid-β and glucagon.
Two examples shown for each protein. Images are taken from the AmyloidAtlas at https://people.mbi.ucla.edu/sawaya/amyloidatlas/ that is described in [6]. Except for glucagon, there are many more structures reported for α-synuclein and amyloid-β amyloids. To relate to the findings described in this text, the examples shown are for amyloids assembled *in vitro* from full-length non-acetylated α-synuclein (**A**) and amyloid-β(1–42) (**B**), respectively. For more amyloid folds adopted by these proteins, and of mutated variants, see the website noted above. (**A**) α-synuclein amyloids: 7yk2 and 6cu8. (**B**) Amyloid-β amyloids: 2nao and 5oqv. (**C**) Glucagon amyloids: 7mx8 and 6 nzn.

Chemical catalysis by glucagon amyloids

Glucagon regulates blood-glucose levels and is involved in lipid metabolism, energy storage and amino acid metabolism. Formation of glucagon amyloids has been reported at many different conditions *in vitro*, but no biological functions of such amyloids are yet known. To address possible catalytic activity of glucagon amyloids, several chemical reactions were assessed *in vitro* [31]. Esterase activity, using pNPA as for amyloid-β amyloids above, was detected for glucagon amyloids but not for the monomeric counterparts (Table 1). Studies of peptide variants revealed that His at position 1 in the 29-residue glucagon peptide was essential for the esterase activity. Next, it was discovered that glucagon amyloids also catalyzed lipid hydrolysis when using para-nitrophenyl palmitate (pNP-palmitate) as model substrate, and dephosphorylation, when using para-nitrophenyl-orthophosphate (pNPP) as a model substrate. Notably, the glucagon amyloids catalyzed dephosphorylation more efficiently with ATP (a real biological substrate) than pNPP as the substrate.

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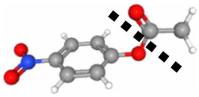
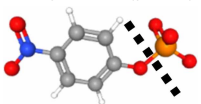
Table 1 Catalytic activity of biological amyloids *in vitro*. Michaelis–Menten parameters for esterase (pNPA) and phosphatase (pNPP) activity of amyloid fibers assembled from α-synuclein, amyloid-β and glucagon polypeptides, along with additional catalytic activities discovered for each amyloid system. n.d., not determined. Chemical structure, with the bond cleaved in the reaction (black dashed line), shown for pNPA and pNPP

Amyloid-forming protein		α-Synuclein[a]	Amyloid-β[b]	Glucagon[c]
Esterase (pNPA) 	K_M	4.3 mM	2.9 mM	4.4 mM
	k_{cat}	0.0127 s^{-1}	0.0019 s^{-1}	0.0025 s^{-1}
	ε	2.9	0.64	0.55
Phosphatase (pNPP) 	K_M	0.5 mM	n.d.	0.12 mM
	k_{cat}	0.0003 s^{-1}	n.d.	0.0070 s^{-1}
	ε	1.7	n.d.	57
Additional catalytic activities		- Alteration (increases as well as decreases) of 21 neuronal metabolite levels[d]	- Acetylthiocholine hydrolysis - Dopamine/adrenaline oxidation	- Lipid hydrolysis - ATP dephosphorylation

[a]From [32]. Conditions: 50 mM phosphate, pH 7.0, 37°C (pNPA), 50 mM Tris, 150 mM NaCl, pH 7.4, 37°C (pNPP;)
[b]From [30]. Conditions: 50 mM HEPES, pH 7.35, 37°;C
[c]From [31]. Conditions: 50 mM HEPES, pH 7.4, 37°;C
[d]Data in [33].

Chemical catalysis by α-synuclein amyloids

Catalytic activity of α-synuclein amyloids was tested on the same model ester pNPA and model phosphoester pNPP substrates as used for amyloid-β and glucagon investigations [32]. It was found that amyloid fibers, but not monomers, of α-synuclein catalyzed hydrolysis of pNPA and dephosphorylation of pNPP with catalytic efficiencies similar to those reported for amyloid-β and glucagon amyloids (Table 1). When His50 was replaced with Ala in α-synuclein, dephosphorylation but not esterase activity of the amyloids was diminished. Thus, synuclein's sole His residue (positioned in the amyloid core in most reported structures, see examples in Figure 1) plays a key role in phosphatase but not esterase activity. In contrast with the other two amyloid systems described, amyloids of the 140-residue α-synuclein protein have long floppy N- and C-termini (typically, N-terminus up to residue 45 and C-terminus from residue 100) protruding from the amyloid core. As an initial test of the role of this 'fuzzy coat', the same experiments were repeated with α-synuclein amyloids made from α-synuclein with the C-terminal 21 residues truncated (α-synuclein(1–119). However, the same catalytic result was observed for these amyloids as found for wild-type α-synuclein amyloids [32].

To assess catalytic activity of α-synuclein amyloids on biological metabolites, we incubated α-synuclein amyloids with neuronal SH-SY5Y cell lysate devoid of proteins [33]. LC–MS based metabolomic analysis unraveled distinct changes in several metabolite levels upon amyloid (but not monomer) incubation for 6 h. Of 63 metabolites that could be identified, four increased (3-hydroxycapric acid, 2-pyrocatechuic acid, adenosine and NAD), and 17 decreased (e.g. several aromatic and apolar amino acids, metabolites in the TCA cycle, keto acids), in the presence of α-synuclein amyloids [33]. Based on the *in vitro* model substrate observations, it was speculated that adenosine and NAD are created via amyloid-catalyzed dephosphorylation of AMP and NADP, respectively. On the other hand, the acids that increased may have been created by esterase activity on precursor metabolites. Even if the involved chemical reactions are yet unknown, many of the detected metabolite changes are similar to those reported in Parkinson's disease patients and animal-models [34,35]. It is important to point out that some of the metabolite decreases detected in the amyloid-incubation study [33] may be a result of amyloid binding. However, it is unlikely to explain the decreases of all 17 metabolites identified.

Structural features that may govern catalytic activity

In contrast with enzymes that have evolved specific geometric shapes of buried catalytic pockets, catalytic amyloids (both designed peptide assemblies and natural pathological amyloids), with active residues protruding from a rigid surface, are expected to be less efficient in reactivity and more promiscuous in terms of substrates.

This is also evident from the catalytic parameters derived (Table 1). Real enzymes evolved to perform esterase and phosphatase activity are orders of magnitude more efficient. However, the catalytic parameters for the three natural amyloids described here are similar to corresponding values reported for short synthetic tailor-made catalytic peptides [26,28,29,36].

The repetitive arrangement of residues on the surface of the amyloids may create weak-affinity substrate-binding arrays along the fiber long axis. Residues such as Lys, Ser, Tyr and Thr, along with His and others, may form catalytic sites [28], in similarity to in small synthetic peptide amyloids [36]. Catalytic triads (nucleophile, acid and base) often form the active sites in enzymes that cleave chemical bonds. Most natural amyloid structures will have Asp and Glu residues (can act as acid), His and Lys residues (acting as base), and Ser and Thr residues (can act as nucleophile) displayed on the amyloid surface (Figure 1). In support of possible altered reactivity, residue pK_a values have been shown to be shifted in amyloid structures as compared with in the monomeric counterparts [37]. Still, inspection of structures of human pathological amyloids does not easily reveal specific catalytic sites; there are many possibilities. It must be stressed that the explanation cannot be simple nonspecific surface-mediated catalysis, as some point mutations in otherwise similar amyloids were found to have dramatic effects on activity (see above). Future studies should clearly target the structure–function correlation, for example by a combination of *in vitro* chemical experiments, protein engineering, computer simulation and docking, and high-resolution structures.

The explosion of high-resolution amyloid structures reported in the last few years makes it clear that the amyloid structural landscape is vast, see e.g. [38,39] and the amyloid collection (with beautiful illustrations) at: https://people.mbi.ucla.edu/sawaya/amyloidatlas/. Amyloid structures formed by different polypeptides, and different amyloid polymorphs adopted by the same polypeptide when aggregated at different conditions, will likely exhibit different catalytic abilities as the surface pattern of reactive residues will vary with the exact fold. There are yet many unknown parameters as amyloid structures determined of amyloid samples from patients differ from the structures found for amyloids created *in vitro* [40,41].

Putative link to amyloid disease progression

Metabolic alterations are linked to neurodegenerative disorders and many metabolites have been evaluated as putative biomarkers for Alzheimer's and Parkinson's diseases [42]. Although altered metabolite levels are mostly considered byproducts of pathology, some studies imply that metabolite accumulation, and their formation of amyloid-like fibers, may be causative agents [43,44].

Studies in animal models of Parkinson's disease, Parkinson's disease patients' tissue and body fluid, as well as cell culture systems challenged with α-synculein amyloids have shown alterations in many metabolic pathways and individual metabolites [34,35]. Increased lipid and fatty acid metabolism have been identified in human Parkinson's disease samples as well as in Parkinson's disease animal and cell models [45–48]. Energy metabolism was affected in multiple studies, with significant decreases in levels of metabolites in the TCA cycle [46,48,49]. Additionally, purine metabolism (including uric acid, hypoxanthine and inosine) has been found affected in Parkinson's disease like conditions [50–52]. Decreased levels of branched chain amino acids, glutamine and aromatic amino acids have also been noted in *in vitro* and *ex vivo* studies of Parkinson's disease [53–56]. Similar metabolite alterations have been noted in Alzheimer's disease [42]. Although many of these metabolite changes may be indirect consequences of amyloid formation, some may be due to catalytic activity of the amyloids themselves. In support, many metabolite alternations observed in patient or animal studies match the metabolite changes we detected upon simple incubation of α-synuclein amyloids with a mixture of neuronal cell metabolites [33].

Metal ion (e.g. iron, copper) dys-homeostasis is linked to neurodegeneration and metal ions often accelerate amyloid formation *in vitro* [16,57,58]. It is possible that amyloids chelate metal ions *in vivo*: such interactions may affect metal ion homeostasis as well as tune the amyloid catalytic activity. Although *in vitro* esterase activity by amyloid-β amyloids was not affected by metal additions [30], metal interactions may play a role for other catalytic reactions in cellular settings. Metal ion interactions with amyloids, *in vitro* and *in vivo*, deserves further investigation due to the clear physiological link between neurodegeneration and iron/copper imbalance.

What is next?

Chemical reactivity of pathological amyloid fibers may be an unexplored gain-of-function in neurodegenerative disorders that promotes alterations in metabolite composition in cells and, thereby, modulates disease progression (Figure 2). To date, however, our understanding of this phenomenon is limited, with only four

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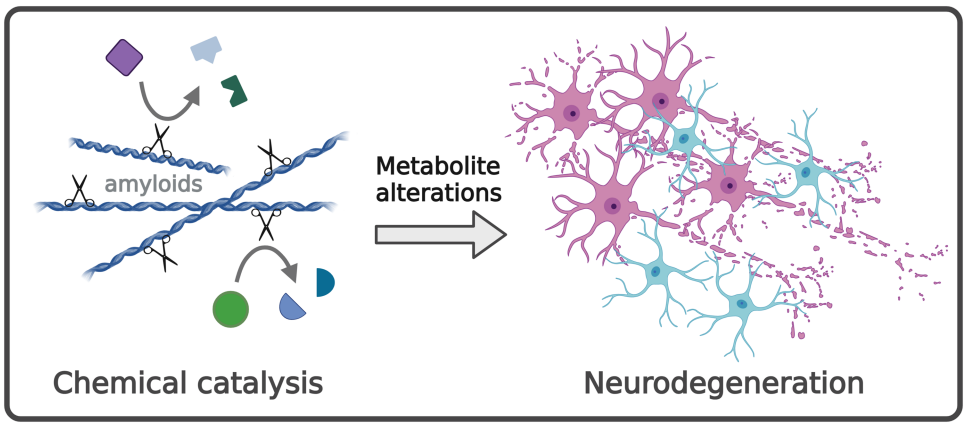

Figure 2. Possible scope of new findings.

Chemical reactivity of pathological amyloids may be an unexplored gain-of-function in amyloid-related diseases (such as in neurodegenerative disorders like Alzheimer's and Parkinson's). Amyloid-mediated chemical catalysis (illustrated by scissors; only active in amyloid scaffold) may promote alterations in metabolite composition in cells and, thereby, modulate disease progression (e.g. accelerate neurodegeneration). Further studies on this emerging topic are warranted. Created with BioRender.com.

publications (reporting on α-synuclein, amyloid-β and glucagon amyloids). To assess the scope of this new amyloid functionality, investigations should assess chemical reactions beyond hydrolysis/cleavage. The possibility that amyloids may promote synthesis (i.e. chemical bond formation) reactions is exciting. In addition to careful *in vitro* studies assessing molecular structure–function relationships for individual substrates and amyloid variants, additional metabolomics experiments should also be designed in which a range of factors are varied. Ultimately, chemical catalysis by amyloids *in vivo* must be proven. This may be achieved by the careful design of high-resolution cell imaging experiments with differentially labeled amyloids as well as substrates.

As a start, I encourage every researcher out there that works on an amyloid system to go into the laboratory and test your amyloid for chemical catalysis. Collectively, we can gather sufficient experimental data to judge the magnitude, and thus the importance, of amyloid chemical catalysis. If amyloid catalysis stands the test, it may provide new ways to understand, as well as attack, human amyloid diseases. It may also be a missed activity of functional amyloids that should be explored.

Perspectives

- Pathological amyloids are the basis of several diseases, such as Alzheimer's and Parkinson's diseases. Most organisms also use amyloid structures to perform biological functions.
- New research challenges the concept of amyloids as chemically inert species. Instead, amyloids act like enzymes and catalyze chemical reactions.
- Catalytic activity of pathological amyloids may be an unexplored activity that contribute to disease progression. All amyloids (functional and pathological) may harbor intrinsic catalytic activity.

Competing Interests

The author declares that there are no competing interests associated with this manuscript.

Abbreviations

pNPA, para-nitrophenyl acetate; pNPP, para-nitrophenyl-orthophosphate.

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