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Protein Aggregates

Amyloids: Not Only Pathological Agents but Also Ordered Nanomaterials**

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Amyloid fibers constitute one of the most abundant and important naturally occurring self-associated assemblies. A variety of protein and peptide molecules with various amino acid sequences form these highly stable and well-organized assemblies under diverse conditions. These assemblies display phase states ranging from liquid crystals to rigid nanotubes. The potential applications of these supramolecular assemblies exceed those of synthetic polymers since the building blocks may introduce biological function in addition to mechanical properties. Here we review the structural characteristics of amyloidal supramolecular assemblies, their potential use as either natural or de novo designed sequences, and the range of applications that have been demonstrated so far.

1. Introduction

1.1. Biophysical Properties

Amyloid fibrils offer an energetically stable alternative state for the functionally folded monomeric state of many proteins and polypeptides. The proteins and peptides that form amyloids undergo a structural transition from native soluble monomeric conformations, in many cases via a natively unfolded intermediate,[1] into aggregative fibrillar assemblies that have predominantly β-sheet structure. Basically, amyloid fibers are a bundle of highly ordered filaments composed of ladders of β strands that run perpendicular to the fiber axis and are arranged in hydrogen-bonded β sheets[2,3] (Figure 1). Amyloid fibers have a characteristic morphology under electron microscopy, with a diameter of about 7–10 nm and lengths of up to few micrometers.[4] In cross sections amyloid assemblies appear as hollow cylinders or ribbons. Recent physical measurements of amyloid fibers revealed that they are comparable to steel in strength and comparable to silk in mechanical stiffness[5] (Table 1).

A typical amyloid fiber is composed of at least two winding protofilament subunits. These generally adopt a cross-β-pleated sheet or a β-helix structure, in which the internal architecture depends on the specific protein species or experimental conditions.[6–8] Protofibrils of protofilaments, the smallest fibrils observed in

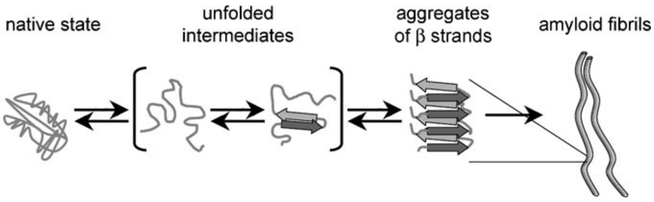

Figure 1. The formation of amyloid fibrils. A natively folded monomer undergoes a conformational transition into a β-sheet-rich state, usually through a partial unfolded state. Self-assembly of these intermediates into ladders of β strands results in the formation of ordered filaments that aggregate into the well-known amyloid fibrils.

Table 1: Comparison of the measured mechanical properties of amyloid fibrils, peptide nanotubes, and spider silk.

	Amyloid fibrils[5,90]	Phe-Phe nanotubes[91]	Spider silk[92–94]
Stiffness [GPa]	–	160×10^{-9}	≤30
Strength [GPa]	0.6 ± 0.4	–	0.9–1
Bending rigidity [Pa]	$(9.1\pm1)\times10^{-26}$	–	–
Shear modulus [GPa]	0.28 ± 0.2	–	2.38
Thermal stability [°C]	≥130	≥150	230
Torsional rigidity [Pa]	$(1.6\pm1.1)\times10^{-26}$	–	–
Young's modulus [GPa]	3.3 ± 0.4	≈19	11–13

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vitro, are reported at the early stages of amyloid formation.[4,9] Amyloids are generated in vivo, both extra- and intracellularly, but can be efficiently and easily reproduced in vitro.[10] Moreover, aggregation into amyloid fibers is not limited to peptide chains of a specific length or having a particular primary or secondary structure; this suggests that the conformational shift into β-sheet-rich structures is energetically preferred.[11,12]

1.2. Role of Amyloids in Diseases

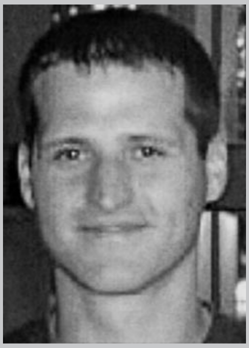
The deposition of amyloid fibrils in various tissues and organs is characteristic of a variety of diseases.[7,13] Among these amyloid-related disorders are Alzheimer's disease, Parkinson's disease, Huntington disease, type II diabetes, and prion disorders. While it is well established that the accumulation of the insoluble protein deposits in the form of amyloids in specific tissues and organs is consistent with these diseases, it has been realized only in the last decade that prefibrillar assemblies may actually represent the toxic elements responsible for the cell death in the infected tissues.[14–22] The toxic oligomer species were observed to have annular, channel-like, or doughnut-shaped structures. Nonetheless, their specific conformation and oligomeric state are still subjects of debate, likewise the exact mechanism of cytotoxicity.

1.3. Amyloids as Self-Assembled Nanostructures

The exact mechanism of amyloid fibril formation, although not fully understood, resembles the processes of crystallization and gelation (Figure 1).[7,23] Amyloid formation begins with the self-assembly of small oligomers that serve as seeds for fibril formation.[11,23] Amyloid fibers are qualitatively different from globular proteins as they much more resemble synthetic polymers: Many amyloidogenic sequences retain their self-assembly properties following amino acid substitutions, truncations, extensions, or specific modifications, sometimes with the result that their β-sheet network is remodeled and fibers of altered morphology are formed.[24,25]

Many studies have been aimed at revealing the nature of amyloidogenic motif sequences to gain insight into the self-assembly mechanism and specifications for the amyloid core.[26,27] Based on these studies, scientists are currently able to design bio-inspired nanostructures based on amyloidal recognition motifs for various applications. In general, amyloid structures attain their stability through noncovalent bonds, notably hydrogen bonds, hydrophobic interactions, and π–π stacking interactions, occurring both between side chains and main chains. The importance of hydrophobic interactions in amyloid fibril formation is rather explicit, since it is principally a process of intermolecular interactions leading to protein aggregation and precipitation. However, the ordered ultrastructure of amyloids suggests that specific patterns of molecular interactions, rather than nonspecific hydrophobic interactions, play a role in the aggregation process. The frequent occurrence of aromatic residues in short amyloid-related peptides suggest that π stacking may play a role in accelerating the self-assembly process that leads to amyloid fibrils.[28,29] π-Stacking interactions may accelerate amyloid fibril formation by providing geometrical restrictions that promote directionality and orientation of the growing fibril, together with energetic contributions stemming from the stacking itself. Indeed, Kim and Hecht[30] showed that replacement of the phenylalanine residues at the C terminus of Aβ(1–42) by hydrophobic residues resulted in somewhat slower aggregation. In addition, a mutant variant containing four phenylalanine residues at the C terminus of Aβ(1–42)) displayed faster amyloidogenesis. High-resolution X-ray and NMR solid-state analyses of amyloid fibers substantiated the contribution of aromatic amino acids to fibril assembly.[31,32] These findings are consistent with the stacking of aromatic rings between layers of adjacent β sheets. Further evidence of the involvement of aromatic interactions in fibril formation is the ability of the phenylalanine dipeptide, an important structural motif of Aβ peptide (Phe19-Phe20), to spontaneously self-assemble into long and stiff nanotubes.[33–35]

The importance of hydrogen bonds is best illustrated by the propensity of glutamine- and asparagine-rich proteins to form amyloids. Extended sequences of repeated glutamine (or asparagine) units are related to several amyloidose diseases, such as Huntington disease and spinocerebellar



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ataxia, and to the aggregation of yeast proteins into prions (e.g. Sup35 and Ure2). Glutamine repeats were suggested to act as polar "zippers" joining molecules together through the propagation of amyloid fibrils.[36] Interestingly, the X-ray diffraction patterns of fibers of the polyglutamine peptide $D_2Q_{15}K_2$ and of the exon-1 peptide of Huntington, having 51 glutamine repeats, are identical, suggesting that the hydrogen-bond "zippers" create a tightly packed, rigid β sheet.[37] Perutz et al. further suggested that polyglutamine or glutamine/asparagine-rich peptides form nanotubular water-filled β-helical structures rather than cross-β-pleated sheets.[38] Sikorski and Atkins[37] challenged this model by suggesting that the $D_2Q_{15}K_2$ molecules fold into a stable β-sheet hairpin-like conformation that self-assembles perpendicular to the hydrogen bonds. Sharma et al.[39] suggested that polyglutamine homopolymers preferably assemble into antiparallel slablike structures, with multiple reverse turns, depending on the length of the polyglutamine expansions.

The self-assembly properties of numerous amyloidogenic motifs, together with their observed polymorphism (or "plasticity", reviewed in Refs. [8,9,40]), makes them attractive natural building blocks for the design of new nanostructures and nanomaterials. Indeed, a recent review by Wetzel et al.[40] emphasized the similarities between amyloids and synthetic polymers and plastics. The key similarities are that: 1) both amyloid and polymer subunits maintain their assembly properties under significant chemical modifications; 2) both assemblies display similar isomorphism by different monomeric units; 3) both monomers display structural polymorphism (i.e., in native structure and polymer structure); 4) in both cases the subunits associate through noncovalent interactions to form a condensed state; 5) both display characteristics of gels or liquid crystals under certain conditions. The main dissimilar features are that amyloidal proteins display unusually specific and complex sequences, allowing utilization or insertion of additional functionality, such as binding sites and catalytic features. In contrast, decoration of synthetic monomer units with high-molecular-weight functional elements may result in polymerization failure or a lower degree of crystallinity in the condensed state. Furthermore, for amyloid fibers, self-assembly following depolymerization (e.g., sonication) is spontaneous and does not require renewed addition of catalysts as in the case of synthetic polymers.

2. Amyloids as Bionanomaterials

2.1. Natural Amyloids

Many proteins in their amyloidal state display exceptional stability, mechanical strength, and increased resistance to degradation; they melt at high temperatures and are more resistant to the presence of sodium dodecyl sulfate (SDS). In addition, highly amyloidogenic proteins, and particularly peptides, are capable of rapid self-assembly. It is hypothesized that owing to these features several microorganisms utilize the amyloid structural motifs in extracellular biomaterials with important physiological roles. The following examples demonstrate how completely different proteinaceous systems developed in bacteria, fungi, and mammals have converged in the course of evolution to produce the same materials with the same ultrastructure to carry out various (or even identical) functions.

2.1.1. Amyloids and Biofilms

The first evidence for the physiological utilization of natural amyloids is the formation of curli fibers (Figure 2). Curli fibers are the proteinaceous component of the extracellular matrix that facilitates the formation of biofilms in many enterobacteria. Curli fibers display the physical and tinctorial characteristics of eukaryotic amyloids.[41–43] The core amyloid domain of curli proteins includes five repeats of 19–23 amino acids each, which are thought to adopt a strand-loop-strand conformation to form compact coils of parallel β helices.[44,45] The amino acid composition of each repeat includes conserved glutamine, asparagine, serine, and aromatic residues, suggested to stabilize the β-helix structure.[43,46] The major curli protein, CsgA, initiates extracellular self-assembly upon secretion from a membrane-bound "nucleator" protein, CsgB. Consequently, curli fibers form a protective coat on the surface of the bacteria, providing them with biotic or abiotic surface adhesion and invasion capacities, resistance to various antimicrobial agents, and pathogenic properties.[47,48] In addition, curli fibers are resistant to protease digestion and treatment with urea, heat, and 1% SDS solution.[49] Importantly, the formation of curli fibers was shown to be critical for biofilm architecture.[50]

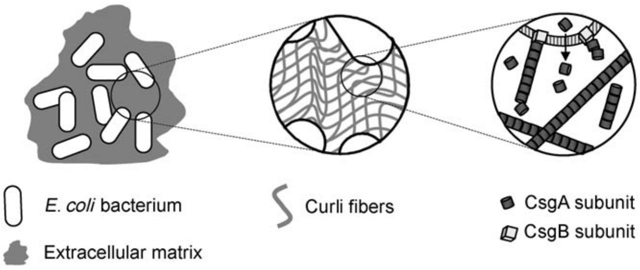

Figure 2. Schematic illustration of the amyloidal curli component of the bacterial extracellular matrix. The formation of the bacterial biofilm (left) is facilitated by the production of a network of curli fibers (middle). CsgA, the major curli subunits, are secreted by bacteria and self-assemble into amyloid fibers extracellularly (right). As a rule, the assembly of curli fibers is initiated by a "nucleator" protein, CgsB, which is anchored to the bacterial membrane.

Curli fibers are a fascinating biomaterial. Besides the protective qualities they endow bacteria, curli fibers exhibit a variety of functional properties encoded in the amino acid sequence. This includes the binding to abiotic and biotic surfaces, cell internalization, and directed assembly from a specific nucleator protein. Such properties may be desired for a range of nanobiotechnological applications.

2.1.2. Amyloids and Hyphae

A class of amyloidogenic proteins all having the same function has been identified on the surface of filamentous fungi and bacteria: class I hydrophobins from fungi, and chaplins from Gram-positive streptomycetes.[51,52] Although they have no evolutionary connection, these proteins have a similar function: they assist branching hyphae (the threads making up the body (or mycelium) of a fungus that usually develop into fruiting bodies) to overcome hydrophobic–hydrophilic interface of water and air. These proteins (consisting of roughly 100 and 50 amino acids, respectively) are secreted to the substrate and self-assemble into an amphipathic monolayer membrane at the air–water interface.[51,52] Consequently, the water surface tension is significantly reduced, allowing the emergence of the hyphae into the air. The surface of the spores is coated by this amphipathic layer as well, leading to better dispersal by wind[53] and enhanced adherence to abiotic and biotic surfaces.[51,52] Hydrophobins self-assemble at oil–water interfaces or on solid surfaces (such as Teflon), thus changing the physical properties of the surfaces. Furthermore, the fibers display resistance to treatment with 2% SDS, heating, and proteolysis.[53]

The amino acid composition of the hydrophobin proteins is considerably diverse, except for eight conserved cysteine residues that stabilize a well-folded β-barrel structure.[54] Chaplins, unlike hydrophobins, polymerize in aqueous solutions containing amyloid nuclei but do not require a hydrophobic–hydrophilic interface.[51] All chaplin proteins contain a hydrophobic region and three conserved glycine–asparagine repeats.[55,56]

The properties of hydrophobins and chaplins make them very attractive candidates for biotechnological applications. The amphipathic nature of the molecules is optimal for the lamination of solid and liquid surfaces, and for changing the physical properties of a surface (hydrophilic to hydrophobic and vice versa). They may be further utilized to improve the biocompatibility of surfaces and serve in the “bottom-up” assembly of scaffolds for cell cultures and tissue engineering. The large variety of natural hydrophobin proteins offers a large pool of biomaterials for fine-tuning specific properties.

2.1.3. Amyloids and Melanin Biosynthesis

Melanin pigments are synthesized and stored in melanosomes, specialized organelles within mammalian melanocytes and retinal epithelial cells. Melanin is formed by the polymerization of reactive indolequinones derived from tyrosine. The maturation of melanosomes requires the production of a transmembrane protein, the glycoprotein Pmel17. Following cleavage, the lumenal fragment of Pmel17 (≈ 80 kDa) rapidly self-assembles into amyloid fibers within the melanosome.[57,58] This fibrillization was demonstrated to accelerate the biosynthesis of melanin since the fibrils serve as templates on which the reactive melanin precursors are polymerized.[57,58] Interestingly, amyloid structures of unrelated proteins could also accelerate melanin polymerization in vitro, suggesting that the function of the Pmel17 scaffold is actually encoded in the ultrastructure itself; most likely the fibrils increase the effective concentration of indolequinone and orient the monomeric units along the fiber.[58,59] The polymerization of recombinant Pmel17, which is four orders of magnitude faster than that of Aβ- or α-synuclein, may have been optimized by evolution to avoid formation of toxic intermediates.[58]

Pmel17 fibers have several unique qualities that are highly desired in the fabrication of new bionanomaterials: 1) the molecules remain soluble until an external trigger (a protease) initiates self-assembly; 2) the polymerization is highly efficient; and 3) the function is displayed only after polymerization. For these reasons, Pmel17 may serve as an interesting scaffold for various technological utilities.

2.1.4 Amyloids and Egg Envelopes

The utilization of amyloid fibers as a protective material was revealed in egg envelopes of several insects and fish.[60,61] The envelope protects the egg from chemical and physical stresses such as temperature variations, mechanical pressure, proteases, bacterial and viral invasion, and dehydration. The major component of the egg envelope is chorion, which is secreted and self-assembles to form the extracellular chorion layer. All of the numerous chorion proteins apparently contain a central conserved amyloidogenic domain consisting of roughly 50 amino acids with hexapeptide periodicities of both glycine and hydrophobic residues (mostly valines).[60,61] The tandem hexapeptide repeats in this domain were suggested to represent the amyloidogenic motif of the chorion proteins.[62] Chorions are a typical example of biopolymers that display the combination of self-assembly and capsulation properties. Integration of chorion domains in biopolymers may provide them with further physical durability.

2.2. A Comparison of Amyloids and Silk

Silk, one of the most thoroughly studied biomaterials, has a broad range of uses, from fabrics to medical applications.[63] The exceptional qualities of both spider and insect silk, which have mechanical properties superior to those of chemical fibers, make them a desirable biomaterial (Table 1). Silk proteins are stored in a soluble state at high concentration inside the silk glands and are converted into silk fibers upon secretion. Each type of silk has a different amino acid composition and exhibits mechanical properties adapted to its specific function.[63] Spider silk proteins are characterized by a highly repetitive primary sequence motif enriched with glycine, alanine, and proline. The hydrophobic patches were suggested to adopt β-sheet structures and form crystalline-like particles (providing strength), while the glycine residues adopt 3_{10}-helical conformations and β turns (providing elasticity); a liquid-crystalline material results.[63–65] Interestingly, silk (mainly spider silk) was found to share some structural characteristics with amyloid fibrils:[64,66] 1) Repetitive primary sequences are frequent among amyloidogenic proteins, bestowing structural features rather than specific recognition or catalytic properties; 2) A transition from a partially (or largely) unstructured state into a stable and irreversible β-sheet-enriched state is evident in both cases; 3) The β-sheet-rich structure of silk is similar to that of many other amyloid fibrils, many of which display an approximate diameter of 10 nm; 4) Recombinant spider silk proteins self-assemble in solution into morphologically similar fibrillar structures. Interestingly, the formation of spherical particles was observed under certain conditions; this has also been reported for amyloidogenic sequences; 5) Spider silk fibrils bind Congo red and ThT amyloid specific dyes; 6) The secondary structures of spider silk and prion fibrils are very similar in terms of the β-structure content. However, amyloid fibrils do not generally contain 3_{10}-helix and/or random coil conformations; and 7) The X-ray diffraction pattern of spider silk and amyloid fibrils suggests a comparable cross-β pattern in which the β sheets are hydrogen-bonded parallel to the fiber axis, but the intersheet packing (5.3 Å) is significantly tighter. Taken together, amyloids and silk fibers might represent structural subclasses with a common structural core.

These examples demonstrate how nature has made use of the remarkable physical properties of amyloids. It appears that current technical applications of amyloidal fibrils represent only a small fraction of their actual potential as a biomaterial.

3. Amyloids in Materials Science

A range of potential technological applications rely on amyloid-fiber-based materials. Amyloids are excellent candidates for the fabrication of molecular nanobiomaterials (e.g., wires, layers, gels, scaffolds, templates, and liquid crystals) using the "bottom-up" strategy. This is because of their structural compatibility, nanoscale dimensions, efficient assembly into well-defined ultrastructures, ease of production, and low cost. Furthermore, the building blocks may be varied extensively by rather simple protein-engineering techniques.

3.1. Amyloid Proteins as Functional Templates

Several studies demonstrated the utilization of amyloids as functional templates (Figure 3). Lindquist and co-workers[67] demonstrated the use of amyloid fibrils as templates for the fabrication of conductive nanowires (Figure 3b). The amyloidogenic protein NM Sup35 (the N-terminal and middle region of yeast prion Sup35), which has known chemical, thermal, and proteolytic stabilities in its fibrillar state, was genetically modified to include a cysteine residue on its surface. Following amyloid formation, colloidal gold particles were covalently linked to the fibrils through the cysteine residues with remarkable specificity; the material was further employed as a template for metalization enhancement with silver ions. The coated fibrils, 80–200 nm in diameter, displayed conductive properties comparable to those of solid conducting wires.

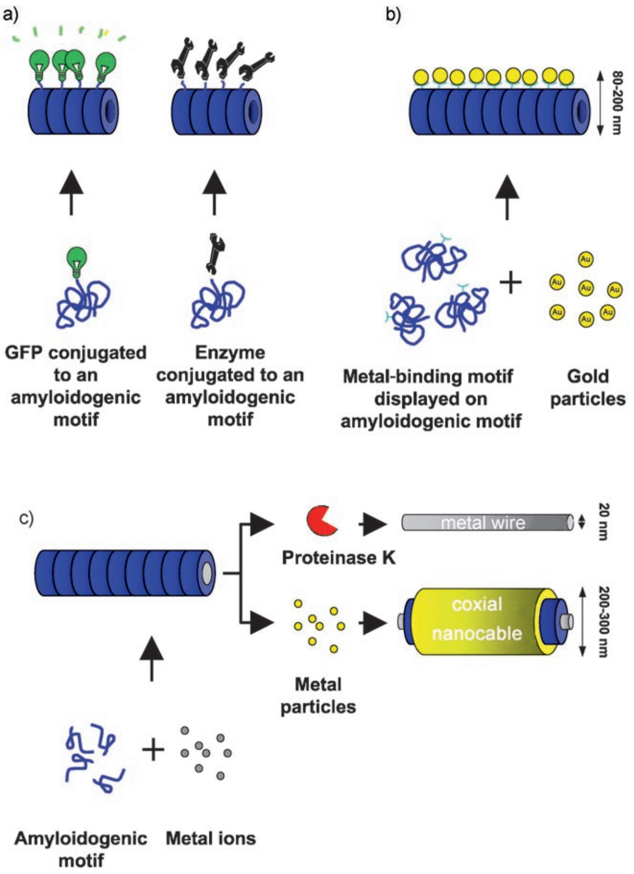

Figure 3. Illustration of amyloids as functional templates. a) Conjugation of functional proteins to certain amyloidogenic motifs resulted in the formation of functional fibers that retain the activity of the conjugated proteins. b) Modification of the amyloidogenic sequence to include a binding motif that is displayed on the surface of the formed fiber can be used to endow it with new functions; in the example shown, displayed cysteine residues bind gold particles to form conducting amyloid fibrils. c) Amyloidogenic motifs forming nanotubes may be used as molding templates for the fabrication of wires within the lumen of the fibers. Degradation of the fiber would result in a nanoscale wire, while further deposition of metal on the activated surface of the fiber would result in a coaxial nanocable.

Perutz et al. suggested that amyloidogenic sequences can form water-filled nanotubes.[38] This architecture might be a favorable scaffold for the fabrication of nanowires (Figure 3c). Lu et al.[69] demonstrated that the KLVFFAE heptapeptide fragment of a β-amyloid polypeptide forms such tubular structures under certain assembly conditions. Nanotubes can also be formed by a smaller fragment of the core recognition motif of β-amyloid. Diphenylalanine (FF) nanotubes have been utilized as a template for metalization.[33] Diffusion of silver ions into the lumen of preformed nanotubes following by enzymatic degradation of the proteinaceous scaffold allowed the fabrication of silver nanowires with a diameter of 20 nm. In another study, this type of dipeptide nanotube was utilized to assemble platinum nanoparticles.[69] In other work, further coating of silver-filled peptide nanotubes with gold resulted in the fabrication of coaxial trilayer metal–peptide–metal nanocables.[70] Recently, FF nanotubes were chemically decorated with biotin moieties and selectively patterned with avidin-labeled species for a range of further applications.[71]

Amyloid fibrils also have potential as a biofunctional material (Figure 3a). Since amyloid sequences tolerate chemical elaboration, a functional protein may be rationally conjugated to an amyloidogenic sequence to endow it with a desirable function. On this basis, Baxa et al.[72] successfully attached green fluorescence protein (GFP), the Barnase bacterial protein, and enzymes such as carbonic anhydrase and glutathione-S-transferase to the C terminus of yeast prion Ure2 monomers and showed that they retained their native structures and remained active after Ure2 amyloidogenesis. Baldwin et al.[73] efficiently attached a functional cytochrome b unit to an amyloid fibril. They then demonstrated efficient incorporation of heme molecules at very high densities on the surface of the formed amyloid fibrils. In that way, they attempted to mimic natural long-distance electron transfer.

3.2. Amyloid-Based Gels and Liquid Crystals

Many polymers, either synthetic or natural, may form gels and/or liquid-crystal phases. Liquid-crystal phases were also observed with amyloidogenic proteins (Figure 4). Corrigan et al.[74] demonstrated that hen lysozyme, known to efficiently form amyloid fibrils at low pH and elevated temperatures, can form liquid-crystal phases composed of a network of lysozyme fibrils. The formed gel actually consisted of a large number of liquid-crystal domains and formed a liquid-crystal glass because of the incapability of the high number of fibrils to align over a long distance. Overall, the formation of the liquid-crystal phases by the solution of hen lysozyme was found to be proportional to the fibril concentration, length, and charge (pH dependence) while rather indifferent to salt presence. Aggeli et al.[75] characterized the gelation properties of the Lysβ-21 peptide, the 41–61 fragment comprising the β domain of the hen lysozyme protein. They found that at higher pH values the Lysβ-21 gel could be transformed into a Newtonian fluid. They further studied the use of pH as a trigger for peptide β-sheet self-assembly into (and out of) β-sheet tapes, ribbons, fibrils, and fibers,[76] thus demonstrating controlled polymerization of monomeric peptides from isotropic fluids up to nematic gel states. This property can be exploited to design self-assembled polymers with controlled mechanical properties and new diverse soft-solidlike nanostructured materials that are biocompatible and biodegradable.

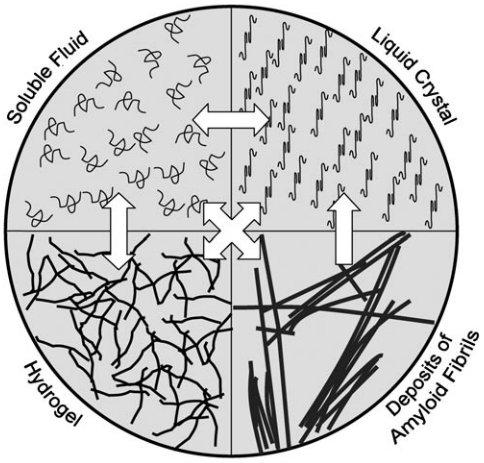

Figure 4. Ultrastructures of phases of displayed by amyloidogenic sequences. The scheme shows the general arrangement of soluble monomers (top left) and assembled fibers (or fibrils) in different possible structural phases. Reported transitions between phases are indicated by arrows.

Recently, the formation of hydrogels from very short peptide conjugate building blocks was reported. The Xu group reported the ability of several 9-fluorenylmethoxycarbonyl (Fmoc) dipeptides and naphthalene(Nap)-Phe-Phe conjugates to polymerize into biocompatible hydrogels.[77,78] The peptide conjugates formed nanofibers that self-assembled into hydrogels held together by a network of π–π and hydrogen-bonding interactions. Also the Fmoc derivative of the β-amyloid diphenylalanine core motif formed a rigid hydrogel, composed of a fibrous network with fibril diameters ranging from 10 to 100 nm.[79] This hydrogel displayed considerable stability at a broad range of temperatures and pH values, in the presence of urea or guanidinium hydrochloride, and under extremely acidic conditions. Compared with other peptide hydrogels, the Fmoc-diphenylalanine hydrogel is remarkably strong and rigid, and thus it may be very advantageous in several applications. For example, it was fabricated into a solid biocompatible mold suitable for encapsulation and controlled drug release, and it is also suitable as a scaffold for cell growth. Recently, the gelation of several Fmoc-dipeptides made up of combinations of glycine, alanine, leucine, and phenylalanine was investigated.[80] Nearly all Fmoc-dipeptides spontaneously assembled into fibrous hydrogels, some of which (including the Fmoc-diphenylalanine) under physiological pH. The amino acid composition was shown to dictate the physical properties of the gels. Most importantly, the researchers showed that certain gels could support two- and three-dimensional cell culture proliferation.

3.3. De Novo Engineering of Amphiphilic β-Sheet-Rich Sequences

Utilization of rationally designed β-sheet peptides, which exhibit amyloid-like characteristics, holds great potential for biomaterial science. The accumulated data regarding the effect of the amino acid composition of amyloidogenic proteins can be used for the de novo design of amyloid-like sequences. Based on the rather simple alternating pattern of polar and nonpolar residues, Hecht and co-workers[81] successfully designed and isolated amphiphilic β-structured sequences that self-assemble into amyloid-like fibrils. They further described self-assembled monolayers and template-directed lamination as applications in protein-based biomaterials. The proteins were shown to self-assemble into β-sheet monolayers at the air–water interface. When deposited on a highly ordered surface of pyrolytic graphite, the proteins assembled into ordered fibers aligned according to the lattice of the graphite surface.

In another study, short amphiphilic β-sheet peptides (7–17 amino acids), composed of repeating pairs of hydrophilic and hydrophobic amino acid residues, were designed to efficiently self-assemble at the air–water interface to form a highly ordered two-dimensional β-sheet crystalline layer.[82] Nonpolar residues were largely restricted to phenylalanine, and proline residues were positioned at the termini to interrupt formation of lateral hydrogen bonds to guarantee regular intermolecular interaction only between juxtaposed β-sheet ribbons. Consequently, the formed layer exhibited exceptional one-dimensional elastic characteristics,[83] providing a planar scaffold pertinent for a wide range of potential applications on a nanometer scale. Potential applications were recently illustrated by Cavalli et al. by the formation of ordered β-sheet lipopeptide monolayers,[84] serving as a template for the biomineralization of calcium carbonate.[85]

Schneider et al.[86] have designed 20-residue amphiphilic sequences that fold into β-hairpins in aqueous solution and further self-assemble into a network of fibrils, leading to the formation of a hydrogel. Upon slight modification of the primary sequences of the peptides, the authors demonstrated that the process could be triggered and reversibly controlled by changes in pH, ionic strength, and temperature, and by UV irradiation.[87] Such a design is likely to be employed in the construction of predictably responsive materials.

Zhang and co-workers have also designed artificial amphiphilic peptides, based on various modules of alternating polar and nonpolar segments, to produce cell culture scaffolds.[88] The peptides, chemically customized for different functions, undergo ordered self-assembly in aqueous solution into roughly 10-nm-wide fibrils and further into a perforated hydrogel with an extremely high water content (> 99 %). For this reason, the peptides were suitable for the fabrication of scaffolds for a range of novel cell growth supports for applications in three-dimensional cell cultures, controlled cell differentiation, tissue engineering, and regenerative medicine.[88,89]

4. Summary and Outlook

It is expected that in the near future further naturally occurring amyloids will be identified, which will substantiate and further establish them as conventional biomaterials. Protein amyloid fibrils have many features that are potentially of great value for the construction of versatile functional advanced materials.

One of the major goals of nanomaterial scientists is to develop new types of polymers suitable for industrial production having desired macroscale structural properties and composed of nanoscale particles with desired functional qualities. Protein and peptide amyloids offer a unique combination of these prerequisites. Apart from being able to integrate biological functionality by covalent fusion or direct patterning, they can assemble into a range of structures from isotropic liquids through different phases of liquid crystals and gels up to solid scaffolds.

In the years to come, further functional applications of either natural or designed amyloidogenic biomolecules will be extensively explored. Such advances may involve the fields of nanobiomedicine and nanoelectronics in the development of sophisticated three-dimensional scaffolds for tissue engineering and regeneration, matrices for controlled drug release and delivery, biosensors and bioswitches, microfluidics devices, coatings for medical or analytical flow devices, biologically inspired catalytic scaffolds, and directed self-assembly of conductive amyloid-based nanoscale wires.

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