1
The Amyloid Phenomenon and Its Significance

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1.1
Introduction

Interest in the topic of ''amyloid'' formation by peptides and proteins has increased dramatically in recent years, transforming it from a puzzling phenomenon associated with a small number of diseases into a major subject of study in disciplines ranging from chemistry and materials science to biology and medicine. The major reason for this explosion of activity is undoubtedly that many of the disorders associated with amyloid formation (see Table 1.1) [1] are no longer rare and somewhat esoteric, as they were even a generation or two ago, but are rapidly becoming the most costly, in terms of health care and social disruption, in the modern world [2]. This change is a consequence of many of these disorders being strongly associated with aging – such as Alzheimer's disease – and with lifestyle and dietary changes – such as type 2 diabetes.

These two diseases alone are having a remarkable effect on our societies, particularly in those countries where life expectancy is now 75 years or more. Thus, for example, the financial burden of Alzheimer's disease in the world each year is already estimated to be approaching $400 billion [2], and the annual cost to the US economy alone is predicted to exceed $1 trillion in 2050 (Figure 1.1) [3]. In addition, type II diabetes is predicted to bring to an end the steadily rising life expectancy in the most highly developed countries [4] and is now becoming widespread amongst the populations of the developing world. And in a quite different context, the recognition of the ability to convert a wide variety of peptides and proteins into filamentous polymers of extraordinary regularity and remarkable properties has opened up opportunities to develop novel self-assembling materials with a range of different characters and potential functions [5] (Chapters 20 and 21).

1.2
The Nature of the Amyloid State of Proteins

The amyloid state of a protein, regardless of its amino acid sequence or the structure of its native state (Table 1.1), is typically manifested in the form of thread like

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Table 1.1 A selection of some of the major human diseases associated with misfolding and the formation of extracellular amyloid deposits or intracellular inclusions with amyloid-like characteristics. Taken from Ref. [1] in which a more comprehensive list is given.

Disease	Aggregating protein or peptide	Length of protein or peptide[a]	Structure of protein or peptide[b]
Neurodegenerative diseases			
Alzheimer's disease[c]	Amyloid β peptide	40 or 42[d]	Natively unfolded
Spongiform encephalopathies[c,e]	Prion protein or fragments thereof	253	Natively unfolded (1–120) and α-helical (121–230)
Parkinson's disease[c]	α-Synuclein	140	Natively unfolded
Amyotrophic lateral sclerosis[c]	Superoxide dismutase 1	153	All-β, Ig-like
Huntington's disease[f]	Huntingtin with long polyQ stretches	3144[g]	Largely natively unfolded
Familial amyloidotic polyneuropathy[f]	Mutants of transthyretin	127	All-β, prealbumin-like
Non-neuropathic systemic amyloidoses			
AL amyloidosis[c]	Immunoglobulin light chains or fragments thereof	ca 90[d]	All-β, Ig-like
AA amyloidosis[c]	Fragments of serum amyloid A protein	76–104[d]	All-β, unknown fold
Senile systemic amyloidosis[c]	Wild-type transthyretin	127	All-β, prealbumin-like
Hemodialysis-related amyloidosis[c]	β2-Microglobulin	99	All-β, Ig-like
Finnish hereditary amyloidosis[f]	Fragments of gelsolin mutants	71	Natively unfolded
Lysozyme amyloidosis[f]	Mutants of lysozyme	130	α + β, lysozyme-fold

Non-neuropathic localized amyloidoses			
ApoAI amyloidosis[f]	Fragments of apolipoprotein AI	80–93[d]	Natively unfolded
Type II diabetes[c]	Amylin	37	Natively unfolded
Medullary carcinoma of the thyroid[c]	Calcitonin	32	Natively unfolded
Hereditary cerebral hemorrhage with amyloidosis[f]	Mutants of amyloid β peptide	40 or 42[d]	Natively unfolded
Injection-localized amyloidosis[c]	Insulin	21 + 30[h]	All-α, insulin-like

[a]Data do not refer to the number of amino acid residues of the precursor proteins, but to the lengths of the processed polypeptide chains that are present in the aggregates that are deposited in the disease states.
[b]This column reports the structural class and fold; both refer to the processed peptides or proteins that deposit into aggregates prior to aggregation and not to the precursor proteins.
[c]Predominantly sporadic although in some of these diseases hereditary forms associated with specific mutations are well documented.
[d]Fragments of various lengths are generated and reported in *ex vivo* fibrils.
[e]Five percent of cases are infectious (iatrogenic).
[f] Predominantly hereditary although in some of these diseases sporadic cases are documented.
[g]Lengths refer to the normal sequences with non-pathogenic traits of polyQ.
[h]Human insulin consists of two chains (A and B with 21 and 30 residues, respectively) covalently bonded by disulfide bridges.

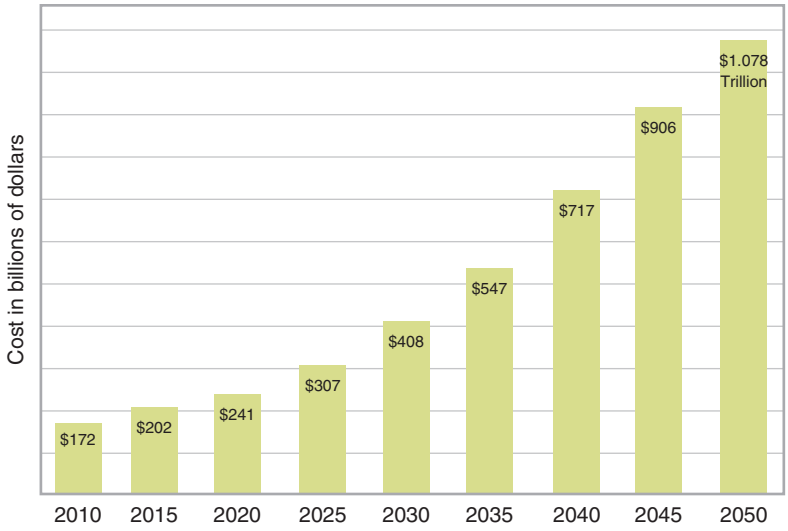

Figure 1.1 Predicted costs for care of people with Alzheimer's disease in the USA. (Source: Alzheimer's Society [3].)

fibrils a few nanometers in diameter and frequently microns in length that are rich in β-sheet structure (see below). In addition to its importance in medicine and materials science, the amyloid state of polypeptides is also of fundamental significance because its existence and properties challenge many of the established concepts about the nature of the functional states of proteins, with their rich variety of distinctive three-dimensional structures, and the manner in which they have been selected through the evolution of life forms and living systems [6]. Thus, for example, experiments with a wide range of peptides and proteins in a laboratory environment has led to the realization that the ability to form amyloid structures is not a rare phenomenon associated with a small number of diseases; instead, the amyloid state emerges as an alternative well-defined structural form that can be adopted under at least some circumstances by many, in principle nearly all, polypeptide sequences [1, 6, 7] (Chapter 14).

Like the native states adopted by globular proteins, amyloid structures are highly close packed and highly ordered, but unlike native states they possess a common or ''generic'' main chain architecture, although the specific details and properties of the structures vary with the composition and sequence of their component amino acid side-chains, as we discuss below. Moreover, there is increasing evidence that the amyloid state might often be more stable than the functional native states of many protein molecules, even under physiological conditions, indicating that the latter may not represent the global energy minima on the free energy surfaces of the corresponding polypeptide chains in living systems but simply metastable states separated from the amyloid form by high activation barriers [8].

A consequence of these findings is that biological systems must have evolved to enable their functional peptides and proteins to remain soluble for prolonged

periods of time under normal physiological conditions rather than converting into the amyloid state, except in the relatively small number of cases where this form of protein structure is utilized for functional purposes, ranging from structural templates to molecular storage devices [1, 9, 10] (Chapters 18–21). Some of these protective mechanisms are undoubtedly encoded in the sequence, notably through the ability of globular proteins to fold into stable and cooperative states, which sequester aggregation-prone regions of the protein in the interior of the molecule and raises the energy barriers to conversion into aggregation-prone species. Indeed, there is evidence that patterns of residues that might favor the amyloid state are commonly selected against during evolution [11, 12] (Chapter 9), and even that the large size of most protein molecules might have the advantage of favoring native folds over the amyloid state [8].

Other protective mechanisms are associated with properties of the cellular environment, such as the existence of molecular chaperones and degradation mechanisms designed to prevent the formation and accumulation of misfolded and aggregated polypeptide chains [13, 14]. Indeed, it is evident that such ''housekeeping'' mechanisms are vital, not just during protein folding following biosynthesis but at all the various stages in the lifecycles of proteins. This conclusion is supported by the discovery of a number of molecular chaperones that act in extracellular space [15], where many proteins in higher organisms function after being secreted from the cells in which they are synthesized, as well as the very large numbers of different types of chaperones that are now known to exist within the cellular environment where synthesis and folding take place [14].

In order to begin to understand the detailed manner in which biological systems are able to ensure that individual proteins adopt the state appropriate to the needs of the organism under specific circumstances (e.g., to enable them to carry out a specific function or to be targeted for degradation) it is necessary to be able to define the nature and properties of the multiple states that are, in principle, accessible to a given sequence [7, 15]. In particular, to understand how living systems are generally able to avoid the conversion of proteins into the amyloid state in contexts where it can cause disease, it is necessary to define the nature of the amyloid structure, the mechanism by which it forms, and the manner in which it can induce pathogenic behavior [1, 7, 16]. In recent years huge strides have been made in each of these areas. Much of this progress has come from the introduction of new techniques and approaches, many of which have been adapted from methods developed for studying molecular systems in other areas of science, for example, microfluidics and nanotechnology [5, 17], as we discuss below.

1.3
The Structure and Properties of Amyloid Species

Unlike the intricate and widely differing structures formed by proteins when folded into their native states, the amyloid forms of proteins look remarkably similar [1, 18]. Amyloid fibrils examined by electron microscopy (EM) or atomic force

Figure 1.2 Model of one of the polymorphs of the amyloid fibrils formed from insulin as defined from cryo-EM analysis. This particular fibril contains four protofilaments that twist around each other to form the mature fibril. Each of the protofilaments has a pair of nearly flat β-sheets, with the component strands oriented perpendicularly to the main fibril axis. See Chapter 15 for more information about fibrillar polymorphism. (Reproduced with permission from Ref. [19].)

microscopy (AFM) are typically thread-like structures that are a few nanometers in diameter but can be microns in length. They are typically composed of a number of protofilaments that twist around each other to form the mature fibril. The core of each protofilament adopts a cross-β structure, in which β-strands are oriented perpendicularly to the axis of the protofilament to form effectively continuous hydrogen-bonded β-sheets running along the length of the fibril (Figure 1.2) [18, 19]. Developments in X-ray diffraction studies of peptide microcrystals [20] (Chapter 2), solid-state NMR spectroscopy [21, 22] (Chapter 3) and cryo-EM [19, 22, 23] (Chapter 4), complemented by other data, for example from real-time small-angle X-ray scattering measurements that provide information about the shapes and populations of different aggregate species present during the process of fibril formation (Chapter 5), have resulted in a steady increase in our detailed knowledge of the molecular structures of amyloid fibrils and reveal that they represent variations on a common theme, as a result of the manner in which the differing side-chains are incorporated into the cross-β fibril architecture that is determined primarily by the properties of the common polypeptide main chain [1, 24]. This generic architecture gives very great stability to the fibrils that are, weight for weight, as strong as steel and highly resistant to degradation by proteolytic enzymes or chemical denaturants [25].

A wide variety of studies has shown that the process of conversion from a soluble, usually monomeric, state of a protein to the polymeric fibrillar state involves a series of elementary steps and a variety of precursor species, commonly including approximately spherical assemblies that are visible in EM and AFM images, and also a variety of shorter protofibrils whose widths are significantly less than those of the mature fibrils (Figure 1.3) [26]. Studies, in particular by mass spectrometry [27, 28] and single molecule optical methods [29, 30], reveal directly that the initial stages of the aggregation process involve the formation of a broad array of oligomeric species. In at least one case that has been studied, that of α-synuclein associated with Parkinson's disease, such oligomers have been observed to undergo a slow transition between relatively disorganized species to more stable structures that are

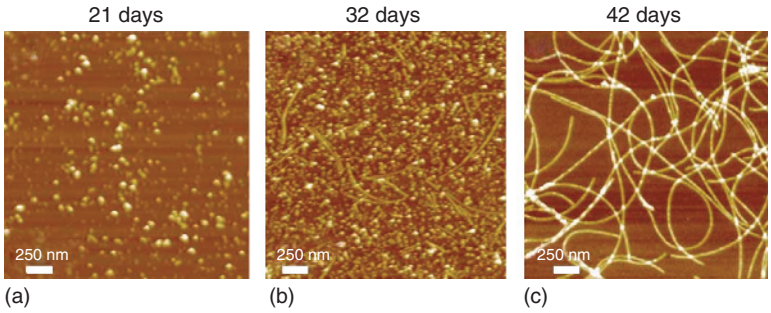

Figure 1.3 AFM images of aggregates formed during the conversion of α-synuclein from its soluble monomeric form into amyloid fibrils. The series of images show the appearance initially of approximately spherical oligomers prior to more numerous aggregates that include thin protofibrils and then mature fibrils. (Reproduced with permission from Ref. [26].)

likely to possess at least a rudimentary cross-β structure and, hence, to be readily able to grow into fibrillar species [30].

1.4 The Kinetics and Mechanism of Amyloid Formation

A major advance in our understanding of the nature of amyloid fibrils has come from careful experimental studies of the kinetics of their formation (Chapter 8) along with the application of mathematical methods that allow the individual rates of the various microscopic processes that underlie the overall aggregation reaction to be defined (Chapter 10). These methods have revealed that the typical sigmoidal curve describing the conversion of a soluble protein into the amyloid structure depends not simply on a single primary nucleation step that is then followed by growth and elongation, but normally also involves at least one secondary process that is dependent on the degree of aggregation that has occurred at any given stage in the reaction (Figure 1.4) [31] (Chapter 10). One well-defined example of such a secondary process is fragmentation, where each breakage event doubles the number of growing fibril ends and, therefore, results in a rapid proliferation of fibrillar species [31, 32].

In addition to this ability to extract the microscopic processes that contribute to the overall aggregation process, developments in microfluidics techniques have enabled additional events to be characterized, including the diffusion of aggregates, a process that represents a crucial step in the spatial propagation of amyloid species [17]. In addition, by varying the volume of the solution in the droplets it has proved possible to observe the primary nucleation step that is essential to initiate the aggregation process and is likely to result in the array of oligomers observed particularly clearly in the single molecule experiments. Such studies also reveal that ''molecular confinement'' within a small volume, such as will occur in a cell

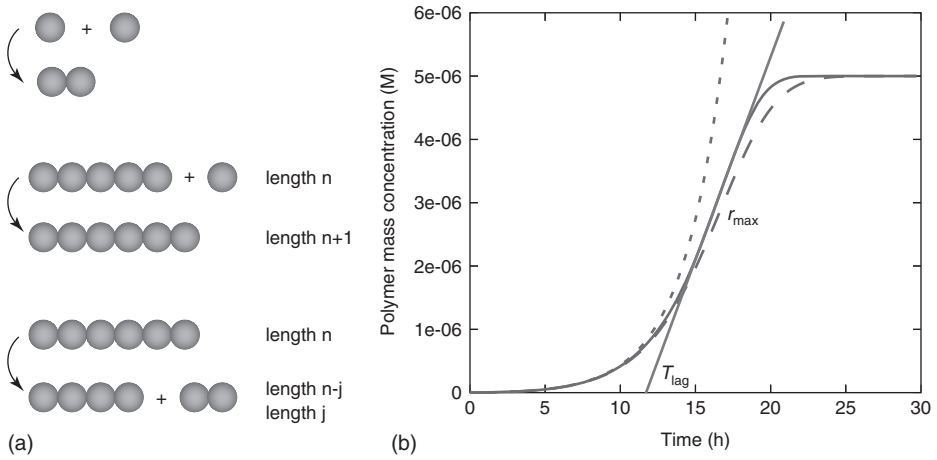

Figure 1.4 (a) Simplified model of the processes involved in the mechanism of formation of amyloid fibrils showing (top to bottom) primary nucleation, growth, and one example of a secondary process (here fibril breakage). (b) A typical sigmoidal curve describing the kinetics of amyloid formation that involves a lag phase, in which primary nucleation occurs, followed by rapid proliferation that is dominated by secondary processes and growth, and then a plateau resulting from the depletion of the soluble form of the protein. Such curves can now be fitted using an analytical expression to extract the microscopic rate constants for the individual steps in the reaction (Chapter 10). (Reproduced with permission from Ref. [31].)

or a cellular compartment, can result in a dramatic reduction in the probability of the initiation of aggregation, hence increasing significantly the kinetic stability of the soluble states of peptides and proteins in biological systems [17].

These structural and mechanistic studies have largely been carried out in the ''test tube,'' and it is of great interest and importance to be able to relate such studies to the events occurring within living systems, and hence to begin to explore the molecular basis of aberrant phenomena that can lead to disease [33, 34]. Exploration of the mechanism of aggregate propagation through analysis of the reaction kinetics can, in principle, be carried out in living systems in a manner analogous to that described above for ''test tube'' experiments, provided that parameters such as the concentrations of the various protein species can be monitored. Indeed, application of such a procedure in studies of transgenic mice provides direct evidence that a dominant contribution to mammalian prion propagation results from fragmentation processes [31].

In addition, studies of amyloid formation within relatively simple model organisms, such as fruit flies and nematode worms, also demonstrate that it is possible to relate the findings of detailed experiments that can be carried out *in vitro* to the events taking place *in vivo* [35] (Chapter 12). There are also opportunities to apply biophysical techniques, for example, those exploiting fluorescence labeling, directly within living systems, and hence to compare the events occurring in the different environments [36] (Chapter 11). Of particular interest in this context is to

understand the ways in which the different types of aggregates interact with the various components of the cell and their consequences for the cellular viability and the onset of disease, as we discuss below.

1.5 The Link between Amyloid Formation and Disease

Although the general link between pathogenicity and the appearance of amyloid deposits in the family of misfolding diseases has been clear for many years, the specific mechanism by which pathogenesis is induced has been the subject of intense debate [1, 37, 38]. For the systemic amyloidoses, the burden of large quantities, literally kilograms in some cases, of fibrillar deposits in vital organs, such as the liver or spleen, is likely to be highly damaging and the major cause of their failure to function normally [1] (Chapter 17). In the case of the neurodegenerative diseases in particular, however, the amyloid burden can be quite low, and there may be little correlation between the quantity of the deposits and the severity of the symptoms [35, 39].

This evidence and the results from a wide variety of experiments over the past decade or so have resulted in the finger of blame, in neurodegenerative disorders at least, being pointed at smaller pre-fibrillar species rather than mature amyloid fibrils [33, 37, 38] (Chapters 6 and 7). Indeed, there is now a mass of evidence that indicates that the oligomeric aggregates discussed above, and which are almost universally observed to be present as intermediates during the interconversion of the soluble and fibrillar forms of peptides and proteins, are ''generically'' damaging to cells; indeed, such cellular toxicity has been observed to be induced by such oligomeric species both for molecules that are associated with disease and those that are not linked to any known pathology (Figure 1.5) [40].

The origin of such toxicity is likely to arise fundamentally from the fact that these oligomers are inherently misfolded, and therefore have the potential to interact inappropriately with many of the functional components of the highly complex and crowded environments with which the latter have co-evolved; the high surface-to volume ratios of these species relative to larger aggregates will also serve to enhance the ability of a given quantity of misfolded material to generate cellular damage [41]. The observation discussed above of an array of oligomers of different sizes strongly suggests that there is no unique ''toxic species'' that is formed during the aggregation process; indeed, it is likely that almost any misfolded species will have the potential to generate at least some level of toxicity [42].

It is increasingly clear, however, that different forms of oligomers can differ significantly in the degree of toxicity that they exhibit [30, 43]. Indeed, it seems likely that the oligomers formed initially during the aggregation process are relatively disordered species whose formation is triggered by the drive to sequester hydrophobic and other aggregation-prone regions of unfolded or misfolded polypeptides away from solvent water molecules. In some cases, at least, it then appears that there is a conformational change associated with the formation of precursors to the

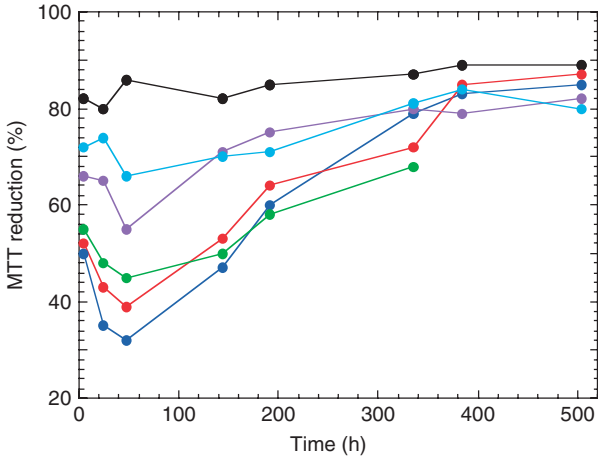

Figure 1.5 Plot of the effects on cell viability (measured using the MTT test) against the length of time that the protein PI3-SH3 had been incubated (during which it converts into amyloid fibrils) prior to the addition of aliquots of the solution to neuronal cells. The different colors represent different volumes of the added aliquots (i.e., the addition of different concentrations of aggregates), with black being the smallest and dark blue the largest volumes. The zero time point represents the monomeric protein prior to aggregation, and the final time points the addition of solutions containing mature fibrils. The aliquots added after 40 h of aggregation contain the highest concentrations of pre-fibrillar (oligomeric) species, as indicated by EM analysis of the solutions. The data show that the oligomeric species are highly toxic, even when formed from a protein with no connection to any known amyloid disease. (Reproduced with permission from Ref. [40].)

amyloid cross-β structure that have both enhanced stability and a more highly hydrophobic surface [30, 41, 44]; such a surface is, however, likely to increase the probability of aberrant interactions with other components of the cell, notably membranes. As these oligomeric species grow in size, and ultimately form mature fibrils and plaques, their surface-to-volume ratios will decrease and many of their more hydrophobic regions may be concealed within the larger assembly, giving rise to a relative reduction in their potential to generate pathological effects.

As discussed earlier in this chapter, the generation of misfolded species with the potential to aggregate is an inherent danger at virtually all times during the lifespan of proteins, and so it is no surprise that these hazardous oligomeric species appear to be a primary target for molecular chaperones, which are likely to target hydrophobic regions on their surfaces, either to induce correct refolding or to sequester the misfolded forms of proteins and to target them for degradation [13, 14, 29]. It will then only be under exceptional circumstances when potentially toxic species are free to generate damage, for example, when the quantities of misfolded and aggregation prone species reach a level where they overwhelm the defensive "housekeeping" systems, leading to cellular malfunction and, ultimately, to apoptosis or other forms of cell death. Of particular interest is the possibility that such a situation can lead to a widespread loss of cellular regulation and a more general breakdown of

proteostasis [13, 45]; the initial aggregation of one protein may, therefore, lead to a range of downstream processes that contribute substantially to the onset of disease.

That such a situation is very likely to be at least broadly correct relates to the fact that proteins can only be selected during evolution only to be good enough for their purpose. It appears from a range of experimental and theoretical studies that many, at least, of our proteins are close to their ''solubility'' limit [46] and, therefore, even small changes – induced, for example, by mutation, post-translational modifications, changes of concentration, or a reduced effectiveness of the protective machinery with age – can be enough to permit self-association to be initiated and then lead to the proliferation of aggregates to the levels that generate pathogenicity (Chapter 9). In this context the growing level of understanding of the aggregation process sheds further light on how such a situation is likely to occur. We can speculate, for example, that chaperones may be very effective at neutralizing the species formed by primary nucleation, particularly before any conversion to amyloid-like forms occurs. But once this stage is passed, secondary process, such as fragmentation and aggregate-dependent catalysis of oligomer formation, coupled with aggregate growth, can give rise to both a rapid rise in the quantities of aggregates and a continuing generation of potentially toxic oligomers [47].

1.6
Strategies for Therapeutic Intervention

Amyloid-related disorders differ from other more well known forms of medical conditions, such as bacterial and viral diseases, or even cancer and heart disease, as they are triggered by the failure of control and regulatory processes to prevent individual protein molecules reverting from their functional states to a persistent misfolded state whose interactions can disrupt the normal processes of life. Pharmaceutical intervention will therefore require different strategies from those applied to ''conventional'' diseases where the selective targeting of specific biological processes, along with other factors such as improved diet and increased standards of hygiene, have proved to be extremely effective in reducing the incidence of disease and also in limiting its effects on the individuals concerned. Ironically, it is advances in the prevention and treatment of these other conditions that has resulted in our increased lifespans that increase dramatically the probability of the onset of amyloid diseases, particularly neurodegenerative conditions such as Alzheimer's disease.

The advances that are being made in understanding the mechanism of aggregation to form amyloid structures, however, offer tremendous opportunities to intervene therapeutically in a rational manner [48, 49] (Chapters 2, 14, and 16). Indeed, there appear to be unique opportunities for such an approach because such therapies need not involve the requirement to affect differentially analogous processes occurring within different types of cells (e.g., bacterial vs human, virally infected vs normal, damaged vs undamaged), but can address the underlying differences between proteins in their functional state and those that are misfolded and which possess fundamentally different properties. Moreover, it is evident that the protective systems that have emerged in biological systems are extraordinarily

effective under the conditions for which they have evolved, notably to enable us to live long enough to pass on our genes and look after our offspring, but then become less effective as we age and become a potential burden for the rest of society. Thus, for example, the occurrence of Alzheimer's disease under the age of 65 is less than 1 in 1000, but at ages above 85 is approximately 1 in 3 [2, 3]. These diseases can be considered to be "post-evolutionary," as they are become epidemic in modern societies where we have effectively moved out of the realm of continuing natural selection [50].

In order to address the treatment or prevention of these diseases it is possible to imagine intervention at different stages of the misfolding and aggregation process, as indicated in Figure 1.6 [48]. There are several general ways in which one can, in

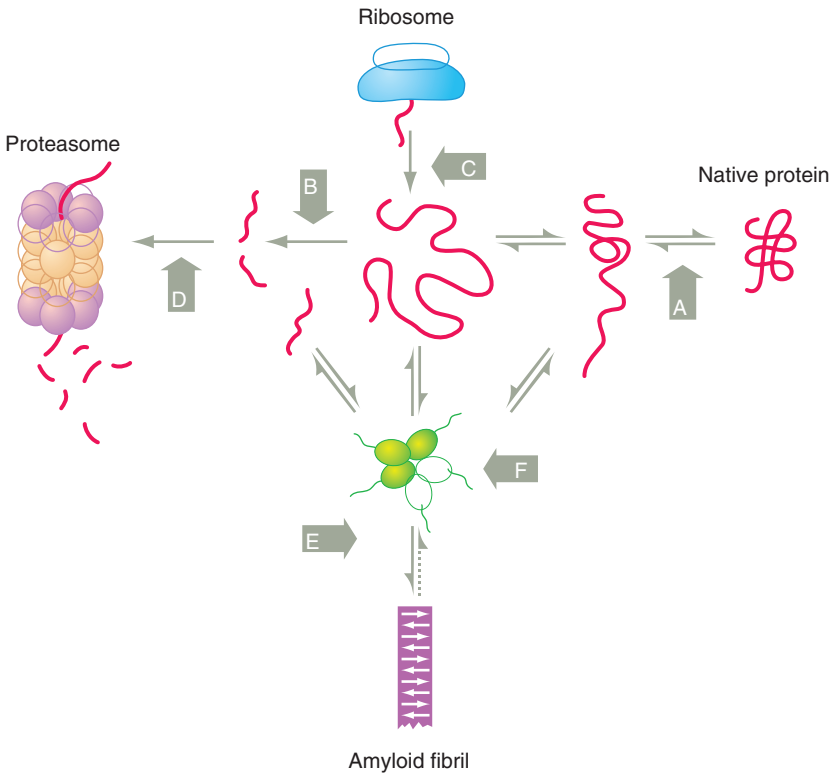

Figure 1.6 Schematic diagram indicating various stages of the lifecycle of a protein in which the propensity to form toxic oligomeric precursors to amyloid fibrils can potentially be inhibited for therapeutic purposes. Therapeutic strategies include (A) stabilizing the native state; (B) inhibiting enzymes that process proteins into peptides with a propensity to aggregate; (C) altering protein synthesis; (D) stimulating clearance of misfolded proteins, for example, by boosting their proteasomal degradation; (E) inhibiting fibril assembly; and (F) preventing accumulation of fibril precursors. (Reproduced with permission from Ref. [48].)

principle, perturb individual steps in the aggregation process, and it is interesting to examine the manner in which biology has generated such effective means of avoiding (at least for most of the ''three score years and ten'' that has traditionally been the maximum lifespan to which any individual might optimistically aspire) the events that lead to a catastrophic breakdown of the ability of organs such as the brain to function.

It is clear that the primary strategy for therapy should ideally be prevention not cure, as once the initial stages of aggregation have taken place the further proliferation of aggregates is likely to be effectively uncontrollable [47]; one excellent example of such a preventative strategy is that designed for the treatment of the amyloid-related conditions based on the aggregation of transthyretin [51] (Chapter 17). This highly β-sheet-rich protein, whose primary natural mechanism of action is to transport the thyroid hormone thyroxin, is a homotetramer in its native state. But this state can be destabilized by a large number of mutations, and the dissociated monomers are highly aggregation prone, giving rise to familial diseases that include both systemic and neurological conditions (Table 1.1). By binding a substrate analog, however, the native state of the protein can be stabilized, thereby reducing the probability of aggregation [51, 52]; indeed this strategy has been developed into a small molecule drug that has now been approved for clinical use.

Another facet of this general strategy is to use the ability of antibodies [53], or artificially generated analogs such as affibodies [54], to bind selectively to the native states of aggregation-prone proteins, as binding generally results in enhanced stability and, hence, to a reduction in aggregation propensity (Chapter 17). In some cases it might be possible to use related approaches to reduce the level of highly aggregation-prone species (such as Aβ1-42) by stimulating clearance [55]. But antibodies and their analogs have other possibilities, one of which could be to mimic the action of natural chaperones by targeting the aberrant misfolded species that give rise to cellular damage. A very exciting step in this general direction has come from the discovery that antibodies can be raised that bind selectively to the oligomeric species that are, as we have discussed above, likely to be the dominant cause of disease, particularly in the case of neurodegenerative conditions [56]. If such ''artificial chaperones'' can be developed, and can be targeted to the appropriate location (e.g., by enhancing their ability to cross the blood–brain barrier), then they could represent a highly effective therapy.

One of the challenges in exploring possible ways of preventing or treating the major neurodegenerative disorders such as Alzheimer's (Chapter 13) and Parkinson's diseases is that the soluble precursors (Aβ and α-synuclein) are not stable globular proteins but, at least under many conditions, they are ''natively unfolded'' or ''intrinsically disordered,'' members of a class of peptides and proteins that is now known to be more common than was previously thought [57]. Strategies based on stabilizing a globular fold are, therefore, not applicable to such situations; but exciting opportunities arise from our increasing knowledge of the conformational properties of such species [58] (Chapter 6) and the increasing

evidence of the importance of the kinetic stability of the functional states of proteins. Thus, for example, it may be possible to maintain the level of toxic oligomeric species below those that can be managed by the cellular ''housekeeping'' mechanisms for longer periods of time and, hence, postpone the onset of disease [48, 59].

1.7 Looking to the Future

There are very considerable grounds for optimism in the ''amyloid field'' both in the opportunities that its further study offers in developing a deep understanding of the nature of functional biological states, and in the quest to prevent or treat the rapidly growing numbers of our populations that are suffering, or otherwise will suffer, from the debilitating diseases with which its formation is related [1–4]. In addition, there will undoubtedly be progress in developing applications in a wide range of fields for these most remarkable self-assembling materials [5]. The increasing evidence for the ''generic'' nature of the amyloid state indicates that it will be possible both to generate a vast range of sequences that are designed to adopt this state and to have a wide range of properties and functionalities. In addition, the ''generic'' factors associated with the mechanism of its formation and the nature and properties of the precursors to the fibrillar state promise to enable common approaches to be adopted to tackle the increasing number of diseases that are now recognized to be associated with its formation [1, 7, 48].

Research into the amyloid phenomenon is at an exceptionally exciting stage. We are beginning to understand at a molecular level the origins of the structures and properties of both the fibrillar and the prefibrillar states of proteins, as well as their functional native states, and the mechanism by which they are formed under different conditions. The enhanced knowledge in both these areas of research provides unprecedented opportunities to perturb in a rational manner the effects of the aberrant behavior of proteins so as to enhance human health and longevity. In order for these advances to be exploited to their full it is important continually to develop rational methods of building on present progress. As we have discussed above, a range of exciting developments is underway, including the introduction of new means of probing the structures of the wide range of amyloid-related states formed from different polypeptide sequences, the exploitation of new techniques for monitoring the microscopic events involved in amyloid formation, such as microfluidics and single molecule methodologies, an enhanced ability to simulate the conformational transitions using advanced computational methods, the development of high resolution imaging techniques that make it possible to monitor the development and fate of aggregates of different types *in vivo* as well as *in vitro*, the extension of a molecular understanding of structure and mechanism from *in vitro* to *in vivo* situations, and the exploitation of new approaches to perturb these processes using both small and large molecules.

In terms of the medical impact of amyloid formation, with such enhanced knowledge and an extension of our understanding of the types of molecules that will interact with, and perturb, the aggregation process, will come novel diagnostics that are desperately needed for detecting the onset and progression of amyloid-associated diseases, for identifying new targets for drug discovery, and for defining the efficacy of therapeutic strategies. To achieve effective and affordable therapies on the timescale that is necessary to avoid a situation where a large fraction of the human race is suffering from highly debilitating and incurable conditions that will place a huge burden on the financial resources of the populations of the world it will be necessary to increase dramatically and rapidly the funds available for research into the amyloid field. At present, for example, the spending on research into amyloid-related diseases is less than one tenth of that directed to cancer, even though the health care cost of just one type of the former, Alzheimer's disease, is much greater than all forms of the latter [2, 3].

With increasing resources should come new ways of carrying out research, that bring together different scientific disciplines, basic scientists and clinicians, theoreticians and experimentalists, and academic and industrial organizations. We need a ''war on dementia'' to match the ''war on cancer'' that came into being with the US National Cancer Act of 1971 [60]. As with cancer, the increasing generation of even rudimentary forms of therapy will make it possible to talk about the disease openly, because diagnosis will cease to be considered a sentence of inevitable decline and untimely death. The chapters that follow, however, reveal the astonishing progress made already in understanding the fundamental process of amyloid formation and its consequences, provide ample evidence for optimism both for controlling the ''amyloid phenomenon'' for medical purposes and potentially exploiting it for the development of new materials and devices for the world of the future.

1.8 Summary

Interest in the phenomenon of amyloid formation by polypeptide molecules has developed with extraordinary rapidity in recent years, such that it is now a major topic of research across a wide range of disciplines. The reasons for this surge of interest arise primarily from (i) the links between amyloid formation and a range of rapidly proliferating medical disorders, including Alzheimer's disease and type II diabetes, (ii) the insights that studies of the amyloid state provide about the nature of the functional forms of peptides and proteins within living systems, and (iii) the opportunities that could exist for generating new therapeutic strategies and for devising new types of materials with novel and potentially useful properties. In this chapter I have given a personal overview of the ''amyloid phenomenon,'' and have discussed some priorities for further investigation and also opportunities for the future. I hope that this chapter serves as an introduction to the remaining chapters in this volume that describe specific aspects of the ''amyloid phenomenon'' in much greater detail.

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