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Pk Nandi

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Review article

Protein conformation and disease

PK Nandi

Pathologie infectieuse et immunologie, Inra, 37380 Nouzilly, France

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Summary — In a number of neurodegenerative diseases, a change in the conformation of specific proteins occurs which is considered to be related to the development of the disease process. In general, a change in the protein conformation from a predominantly α -helical to a predominantly β -sheet structure, due to genetic or environmental factors, leads to oligomerization of the proteins. In Alzheimer's and prion diseases, insoluble precipitates or plaques are often observed in vivo as a consequence of β -fibril formation due to the polymerization of the proteins. In certain other neurodegenerative diseases, for example in Huntington disease, the β -sheet oligomers may interact differently with other functional proteins compared to the monomeric proteins inducing the disease. Biophysical studies would provide information regarding the forces responsible for the oligomerization of these proteins and hence provide methods for detecting these diseases, leading eventually to therapeutic approaches.

protein conformation / neurodegenerative disease / Alzheimer / β -protein / prion protein detection / therapy

Résumé — **Conformation des protéines et maladie.** *Dans un certain nombre de maladies neurodégénératives, un changement dans la conformation de protéines spécifiques se produit, que l'on pense être lié au développement de ces maladies. En général, un changement dans la conformation de la protéine, passant d'une conformation avec prédominance d'hélice- α à une structure en feuillet- β , que ce changement soit dû à des facteurs génétiques ou environnementaux, conduit à une oligomérisation des protéines. Dans la maladie d'Alzheimer et les maladies à prions, des précipités insolubles ou des plaques sont souvent visualisées in vivo comme une conséquence de la formation de fibrille β , due à la polymérisation des protéines. Dans certaines autres maladies neurodégénératives, par exemple la maladie d'Huntington, les oligomères en feuillet- β pourraient interagir de manière différentielle avec d'autres protéines fonctionnelles, comparé aux protéines monomériques qui induisent la maladie. Des études biophysiques pourraient fournir des informations concernant les forces responsables de l'oligomérisation de ces protéines, et, de là, fournir une méthode de détection de ces maladies conduisant à des approches thérapeutiques.*

conformation des protéines / maladie neurodégénérative / Alzheimer / protéine β / détection des protéines prion / thérapie

INTRODUCTION

After its synthesis from the ribosome inside the cell the nascent protein normally takes up an extended random coil structure. The biological function of a protein depends on its ability to assume a specific ordered conformation. Inability to take up this particular conformation, due to mutations or environmental factors, hampers the protein's ability to carry out its normal biological function and may produce a disease in animals and humans. For certain proteins, the mutation and environmental factors may induce a different conformation which may also produce a disease and result in the ultimately death of the affected individual. Proteins synthesized in the cell are normally a mixture of α -helix, β -sheet, β -turn, β -helix and random coil structures. For a given protein, an increase in the quantity of β -sheet structures at the expense of α -helices, due to the above factors, results in a variety of diseases including neurodegenerative ones. This article reviews the relation between the structural changes of relevant proteins and specific neurodegenerative diseases.

ALZHEIMER'S DISEASE

The deposition of amyloid plaque, a kind of fibrous protein aggregate, is characteristic of many diseases. This includes several forms of systemic (hereditary cerebral angiopathy with amyloidosis, eg, Dutch type, Down's syndrome, sporadic cerebral amyloid angiopathy and normal aging) and localized conditions such as Alzheimer's disease (AD) and type II diabetes (Castano and Frangione, 1988; Frangione, 1989; Cohen and Skinner, 1990; Selkoe, 1991; Yanker and Mesulam, 1991). Together these diseases have been termed the β -amyloid-related diseases.

AD is the most common type of senile dementia (table I). It is manifested by a grad-

ual loss of memory and the progressive deterioration of higher cognitive functions and behaviour (Price et al, 1993). These symptoms result from neural abnormalities in various regions of the brain and in neural circuits, leading to a loss of sympatic inputs in the amygdala, hippocampus and neocortex (De Souza et al, 1986; Braak and Braak, 1991). AD lesions are generally characterized by the presence of β -pleated amyloid deposits which occur predominantly in the hippocampus and neocortex (Goedert et al, 1993). Amyloid fibrils (8 nm long) are composed of a 4 kDa amyloid β -protein derived from larger amyloid precursor proteins (APP), which are coded by a gene in chromosome 21 (Kang et al, 1987). The precursor protein is an integral membrane glycoprotein appearing at the cell surface; the majority are endoproteolytically cleaved (Weidemann et al, 1989; Price et al, 1993). Several APPs of different lengths have been found that are derived from the alternative splicing of a single gene in chromosome 21 (Kang et al, 1987; Robakis et al, 1987; Goldgaber et al, 1987; Tanzi et al, 1987). In autosomal-dominant familial AD patients, a missense mutation in the precursor protein takes place, the valine being replaced by isoleucine, phenyl alanine or glycine (codon 717 of precursors 770 and 693) in the transmembrane region of the protein (Groate et al, 1991; Chatlier-Haslin et al, 1991; Murrell et al, 1991; Naruse et al, 1991; Price et al, 1993). A double mutation at codons 670 and 671, involving substitution of the normal Lys-Met dipeptide by Asn-Leu, has been related to early onset of AD in affected families in Sweden (Price et al, 1993). The major cause of early onset of AD has also been attributed to chromosome 14 (Schellenberg et al, 1992).

The amyloid protein which accumulates in patients with Finnish amyloidosis is different from all other amyloid proteins described above. This amyloidosis is clinically characterized by corneal lattice dys-

trophy, progressive cranial neuropathy and skin changes, as well as renal and cardiac manifestations (Murray and Nurmiaho-Lassila, 1992). The amyloid protein of this type of amyloidosis shows amino acid sequence homology with a variant of the cytoskeletal gel-solin protein (Ghiso et al, 1990). This amyloid protein is a 71-amino-acid-long polypeptide derived from an Asp \rightarrow Asn mutation, at codon 189 (point mutation G \rightarrow A), by limited proteolysis (Ghiso et al, 1990; Wisniewski et al, 1991; Murray and Nurmiaho-Lassila, 1992).

The amyloid precursor protein is abundant and widely distributed in the body (Wisniewski et al, 1991). The process by which the formation of fibrils and senile plaque occurs is still unresolved. Extracellular and

cerebrovascular plaques are primarily composed of variants of the 4-kDa β -amyloid protein (AD) which differ only at the C-terminus. Neuritic plaque has been found to contain the variant β 1-42 (42 amino acids) whereas cerebrovascular plaque contains β 1-39 or β 1-40 peptides (Joachim et al, 1988; Mori et al, 1992; Miller et al, 1993; Jarrett et al, 1993). The precursor molecule on hydrophobicity plots is hydrophilic and contains several α -helical sequences. In contrast, the amyloid β -protein derived from this precursor has predominantly (90%) β -sheet structure, which makes it highly insoluble with a tendency to precipitate in vivo (Joachim et al, 1988).

Analysis of the amino acid sequence of the Alzheimer 43-kDa amyloid β -peptide

Table I. Characteristics of AD and prion disease (from Price et al, 1993)

	<i>Alzheimer's disease</i>	<i>Prion disease</i>
Onset	Mid-to-late life (usually late)	Any age (usually later in life)
Progression	8–12 years	6 months to 8 years
Signs	Dementia	Dementia, ataxia, myoclonus
Sporadic	Majority	Majority
Transmissible	Not documented	Well documented
Familial	5–10 %	Well documented
Chromosomal loci	21, 14, 19	20
Gene with mutation	<i>APP</i>	<i>PrP</i>
Vulnerable cells	Cortical, hippocampal, cholinergic basal forebrain neurons	Many regions of brain
Cytoskeletal pathology	Neurofibrillary tangles, neurites neuropil threads	Rare
Amyloid	A β	PrP ^{sc}
Cell death	Severe	Variable

(Ab) by a different algorithm suggests that the hydrophobic C-terminal domain (residues 29–42) can adopt a β -strand conformation whereas the N-terminal domain (residues 10–24) displays a dynamic equilibrium between α -helices and β -strands. The single mutation of valine 18 to alanine induces a significant increase in the α -helical content of A β as determined by Fourier transform infrared spectroscopy (FTIR) and circular dichroism (CD), and dramatically diminishes fibrillogenesis (Soto et al, 1995). In the hereditary Dutch cerebral hemorrhage disease with amyloidosis (a variant of AD), a decrease in the propensity of the A β N-terminal domain to adopt an α -helical structure is observed when glutamic acid at position 22 is substituted by glutamine; this substitution concomitantly reveals an increase in amyloid formation (Soto et al, 1995). Based on these experiments, it has been proposed that when the N-terminal domain of the A β assumes a β -strand conformation, it can form amyloids. This is similar to the hypothesis proposed for another type of amyloidosis, where the conversion of an α -helix of a prion protein to a β -strand produces the pathology of prion-related neurodegenerative diseases.

PRION DISEASES

Prion diseases (PD) are a group of neurodegenerative disorders found in animals and humans (table I). Scrapie of sheep and goats is the prototypical prion disease (Hunter, 1992). Currently six diseases of animals and four of humans have been identified as being caused by prions (Prusiner, 1992, 1994). Mink encephalopathy, chronic wasting disease, bovine spongiform encephalopathy (BSE, 'mad cow disease'), feline spongiform encephalopathy and exotic ungulate encephalopathy most probably occur after the consumption of prion-infected foodstuffs. BSE has caused a great deal of

concern in the dairy and meat industries in Great Britain. Human prion diseases include the spongiform encephalopathies Kuru (occurring in the Fore-tribe population of New Guinea), Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS) and familial fatal insomnia: these can occur in victims of any age (Price et al, 1993; Prusiner, 1994). PD can often be transmitted to experimental animals by inoculation (Hsiao et al, 1994). The diseases display a remarkable array of clinical abnormalities depending on various factors. These abnormalities include cognitive impairments, motor dysfunction, myoclonus and ataxia which result in spongiform change, neuronal loss and gliosis, particularly in the hippocampus, neocortex, subcortical nuclei, cerebellum and brainstem.

Unlike other transmissible disorders, a mutation of the prion protein can also induce the prion disease. The single-copy *PrP* gene is located in the chromosome 20, and specific clinical symptoms result from specific mutations in the gene in this chromosome (Prusiner, 1994). In some families infected with CJD, the families have point mutations at codons 178 (Asp \rightarrow Asn) or 200 (Glu \rightarrow Lys) (Goldfarb et al, 1990; Brown et al, 1992; Medori et al, 1992). In the case of familial fatal insomnia, a mutation at codon 178 has been identified. In families with GSS, mutation takes place at codons 102, 117, 198 and 217 (Goldgaber et al, 1989; Hsiao et al, 1991, 1992). In one related GSS, prion aggregates as well as senile plaques and neurofibrillary tangles have been observed (Ghetti et al, 1989; Hsiao et al, 1992).

A constant feature of prion diseases, based on the 'protein only' hypothesis, is the synthesis and accumulation of the protease-resistant scrapie isoform PrP^{SC}, the principle component of the amyloid plaques observed in vivo (Price et al, 1993; Prusiner, 1994). The normal cellular prion, PrP^C, is a sialylated glycoprotein of yet unknown bio-

logical function (Prusiner, 1994; Caughey and Raymond, 1991). In prion disease, transmitted or infectious, PrP^C is converted by a post-translational process into the abnormal isoform PrP^{SC}. These results have been obtained principally from neuroblastoma cell lines. Both the prion isoforms have a molecular mass of 33–35 kDa. Peptide mapping and mass spectra have not revealed any difference between their amino acid sequences and that predicted from the *PrP* gene sequence (Stahl et al, 1993). In addition, no chemical modifications have been detected which might distinguish between the two isoforms. However FTIR and CD spectroscopy demonstrated a substantial conformational difference. Normal PrP^C is essentially α -helical (42%) with little or no β -sheet (3%), whereas PrP^{SC} has a high β -sheet content (43%) (Pan et al, 1993; Safar et al, 1990, 1994). Partial proteolysis of PrP^{SC} generates PrP-27-30 which forms amyloid pluck in vitro and has an even higher level of β -sheet content (54%), consistent with its polymerization into amyloid (Caughey et al, 1991; Gasset et al, 1992; Pan et al, 1993; Nguyen et al, 1995). The post-translational conformational modification may take place either in the endosomal compartment or in the segregated region of the plasma membrane which is rich in cholesterol (Stahl et al, 1993; Shyng et al, 1994). It has been suggested that disease-linked mutations in PrP cause conformational changes that promote its spontaneous transformation into amyloid. The presence of amyloid plaques, however, is not obligatory for the identification of a prion disease. Goldfarb et al (1993) studied the synthetic polypeptides corresponding to the sequence encoded by normal PrP and by PrP with mutations in codons 178 and 200, which have been linked to familial CJD. The peptides corresponding to the mutant sequences are more fibrilogenic than their normal counterparts; in addition the morphology of codon 178 fibrils differs from that of the codon 200 fibrils and the mutant pep-

tides accelerate the amyloidogenesis of normal peptides. In a detailed study using synthetic peptides homologous to consecutive segments of GSS-IK amyloid protein as well as peptides from the PrP region with GSS-IK mutation, Tagliavini et al (1993) suggested that the sequence spanning residues 106–147 of PrP is central to amyloid fibril formation in GSS and related encephalopathies. The most fibrilogenic peptide corresponded to residues 106–126. The application of this peptide to rat hippocampal cultures caused neuronal deaths by apoptosis (Forloni et al, 1993). However, the peptides 109–122, which spontaneously form β -sheet fibrils, have failed to induce prion disease in Syrian hamsters (Gasset et al, 1992, 1993; Nguyen et al, 1995).

The transmissible nature of prion diseases has also been ascribed to virus-induced amyloidoses (Diringer, 1992; Rohwer, 1991). In fact, a small virus-like structure in fractions from hamster scrapie has been reported (Ozel and Diringer, 1994). The evidence in the literature is generally more supportive of a 'protein only' hypothesis for prion disease, although the prion model does not adequately account for the strain variations observed.

OTHER NEURODEGENERATIVE DISEASES

Five predominantly inherited neurodegenerative diseases, viz Huntington disease, spinal and bulbar muscular atrophy (Kennedy disease), spinocerebellar ataxia type 1, dentatorubral-pallidolusian atrophy and Machado–Joseph disease (MJD), become apparent in the victims during the third or fourth decade of their lives (Stott et al, 1995). In MJD for example, the symptoms are cerebellar ataxia, pyramidal and extrapyramidal signs, peripheral nerve palsy, external ophthalmoplegia, facial and lingual fasciculation and bulging eyes (Kawaguchi

et al, 1994). An increase in the glutamine content of the relevant proteins has been related to the diseases. The glutamine repeats are coded by CAG trinucleotides. The expansion of CAG leads to a lengthening of the repeat in successive generations of affected individuals.

Poly(-L-)glutamine forms a β -structure in solution. Incorporation of glutamine residues ($n = 10$) into truncated monomeric chymotrypsin inhibitor from barley seed induces oligomerization of the recombinant protein. The CD spectra of the recombinant protein compared to the wild-type protein indicates that incorporation of glutamine repeats induces more β -structure. Extension of their glutamine repeats may cause the affected proteins to aggregate due to their increased β -sheet structure, ultimately leading to precipitation in the neurons (Perutz, 1994). It has also been shown that neural damage in diseases affected by expanded glutamine repeats is due to gain rather than loss of function. The androgen receptor responsible for Kennedy disease is a transcription factor. Increased glutamine repeats would cause oligomerization of the protein due to increased β -sheet structure, which would in turn cause abnormal transcriptional activity (Stott et al, 1995).

STRATEGIES FOR THE DETECTION OF THE ONSET OF NEURODEGENERATIVE PD DISEASES

I will focus on the possible detection of the onset of PD in animals and humans based on conformational changes in the proteins which lead to PD. The term 'onset' refers to a preclinical diagnostic test which would identify the disease in an incubation phase in the individual. There are currently no acceptable techniques to determine whether an apparently-healthy animal (or human) may be incubating the disease. In the case of PD, there is no immune or inflammatory

response in the sick individual and bioassay (transmission to other animal) is the only way to detect scrapie in animals. Diagnosis of PD is based on clinical signs, post-mortem brain pathology and postmortem biochemistry. Results from biochemical markers attempting to detect PD during its incubation are contradictory.

The term 'incubation', refers to the fact that the affected individuals, due to their genetic, environmental or metabolic preindisposition, express the scrapie isoform PrP^{Sc} on their cell surfaces, but in quantities insufficient to result in amyloid plaque formation in the cytoplasm (in the endocytotic compartments) which would then lead to cell death. However, due to the very nature of the PrP^{Sc} β -sheet structure, it has a tendency to oligomerize on the cell surface microdomain either in the clathrin-coated pits or in caveoli that are rich in cholesterol. The normal PrP^C is metabolized in the endocytotic pathway inside the cell (Taraboulos et al, 1995). A diagnostic strategy needs to be aimed at determining the presence of oligomeric PrP^{Sc} on the cell surface of the incubating individual. The procedure should be extremely sensitive for the detection of low levels of PrP^{Sc} expression on the cell surface. The expression of prion PrP^C has been detected in bovine, sheep and human lymphocytes. Density-gradient fractionation of splenic cell populations of scrapie-infected mice revealed infection in all the cell fractions (Annual Report, Institute for Animal Health, Agricultural and Food Research Council, UK, 1993). As yet, PrP^{Sc} has not been detected in any cells other than cell lines (see above). This may be due, however, to the current unavailability of an appropriate antibody. Since antibodies, in general, recognize both sequential and conformational epitopes, attempts should be directed towards developing antibodies capable of detecting the PrP^{Sc} oligomer.

At present, the antibodies which have been developed against PrP^{Sc} recognize

both PrP^C and PrP^{SC} (dissociated) to similar extents (Safar et al, 1990). Recently a limited number of antibodies developed against certain synthetic prion peptides (which can adopt both α -helix and β -sheet conformations) have been shown to differentiate to a certain extent between normal and scrapie isoforms in vitro (Schmerr, 1994). These peptides however rapidly form β -sheet structures and polymerize to amyloid plaque in vitro. Therefore, a strategy which would keep these peptides in oligomeric form would be useful for the production of antibodies that could be used for the diagnosis of oligomeric PrP^{SC} on the surface of cells expressing PrP^{SC}.

The prion peptide sequence 106–147 has been identified as being central to amyloid formation in GSS and related encephalopathies. The sequence is:

106

Lys Thr Asn Met Lys His Met Ala Gly Ala
Ala Ala Ala Gly Ala

Val Val Gly Gly Leu Gly Gly Tyr Met LeuGly
Ser Ala Met

Ser Arg Pro Ileu Ileu His Phe Gly Ser

147

Asp Glu

The peptide fragments 106–126 and 127–147 form respectively straight fibrils (similar to those extracted from GSS brains) and twisted fibrils (resembling scrapie-associated fibrils) (Tagliavini et al, 1993). The amount of ionic charge present is not sufficient to keep these peptides from polymerizing, due to hydrophobic and amide interactions in a nonpolar environment. Incorporation of excess charge by coupling the charged amino acids to one or both ends of these peptides would stabilize the oligomeric forms of the peptide due to repulsive forces.

Another possible technique that can be envisaged to limit the degree of polymerization and keep the peptide(s) in oligomeric form, could be to insert the peptide or its most relevant portion into a biochemical support, for example a protein with excess positive or negative charge (and hydrophilic groups). The support protein could be of low molecular weight, for example, a 93-amino-acid barley chymotrypsin inhibitor (C12) with four positive charges overall (Stott et al, 1995). Insertion of the above prion peptide (pp) gene sequence into the plasmid pC 12, which has been used to express the recombinant inhibitor, would allow the expression of the hybrid protein (C12-pp) with four positive charges. It could then be expected that the hybrid protein would remain as an oligomer in solution with the prion peptide sequence. Evidence has recently been presented that a polyglutamine sequence, which by itself forms β -strands and polymerizes, induces increased β -structure and oligomerization in the hybrid protein when incorporated in the above recombinant monomeric enzyme (Stott et al, 1995). Since antibodies, in general, recognize sequential peptides (segmental) and conformational epitopes (assembled topographic sites), the antibody(s) developed against oligomeric C12-pp (or C12 hybrids with other relevant prion peptides) stand a good chance of being able to detect oligomeric PrP^{SC} on the cell surfaces of the incubating individual, animal or human.

THERAPEUTIC INTERVENTION

It is conceivable that an appreciation of the molecular processes underlying the conversion of α -helix to β -sheet structures would be helpful for the design of new therapeutic strategies for inhibiting the development of the neurodegenerative diseases described here, since they are predominantly caused by the aggregation of specific proteins. Bio-

physical studies would be useful as they could lead to the development of methods for: (i) blocking specific aggregation by designing competitive inhibitors of the aggregation process; and (ii) preventing aggregation, which is a second (or higher) order reaction, by lowering the concentration of the intermediate steps necessary for the formation of amyloid deposits. The former has been considered as a possible therapeutic approach for AD. Although the β -pleated sheets are characteristic of amyloid plaques, the fibrils cross- β -form, arising from quaternary structure (predominantly hydrophobic forces), could be made a target for the prevention of plaque aggregation. A hydrophobic drug, anthracycline 4'-iodo-4'-deoxydoxorubicin, is expected to prevent aggregation in the early phase of fibril formation (Merlini et al, 1995). This drug has produced unexpected and significant benefits in patients with immunoglobulin light-chain amyloidosis (Merlini et al, 1995). The favourable interaction of the amide dipole (δ^-)O=C-NH (δ^+) with the iodide ion has been well demonstrated (Nandi and Robinson, 1972). The covalent iodide of the drug, with its unshared electrons, can interact with the amide dipole (weak electrostatic interaction, a phenomenon recently identified in proteins). A combination of hydrophobic forces and this interaction would probably interfere with the formation of the fibrils. This approach could be refined and new products developed to treat neurodegenerative diseases, including prion.

CONCLUSION

Increasing evidence is becoming available which indicates that a change in the conformation of specific proteins from α -helical to β -sheet structure is related to many neurodegenerative diseases. Formation of β -sheet structure causes oligomerization and polymerization leading to the formation of

amyloid plaques in several neurodegenerative diseases. Intriguing similarities between prion diseases and Alzheimer's disease may suggest a common mechanism of pathogenesis in these illnesses. Understanding the forces responsible for oligomerization may help identify the diseases and assist their prevention.

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