β-Amyloid Protein in Alzheimer's Disease

Josephine Nalbantoglu

ABSTRACT: β-amyloid protein, a 42-43 amino acid polypeptide, accumulates abnormally in senile plaques and the cerebral vasculature in Alzheimer's disease. This polypeptide is derived from a membrane-associated precursor which has several isoforms expressed in many tissues. The precursor protein is processed constitutively within the β-amyloid domain, leading to the release of the large N-terminal portion into the extracellular medium. β-amyloid protein may be toxic to certain neuronal cell types and its early deposition may be an important event in the pathogenesis of Alzheimer's disease.

RÉSUMÉ: La protéine β-amyloïde dans la maladie d'Alzheimer. La protéine β-amyloïde, un polypeptide composé de 42-43 acides aminés, s'accumule de façon anormale dans les plaques séniles et le système vasculaire cérébral dans la maladie d'Alzheimer. Ce polypeptide est formé à partir d'un précurseur associé à la membrane cellulaire, dont plusieurs isoformes sont exprimées dans différents tissus. La protéine qui sert de précurseur est formée à l'intérieur du domaine β-amyloïde, entraînant la libération dans le milieu extra-cellulaire de la portion N-terminale de grande taille. L'accumulation de la protéine β-amyloïde peut résulter d'un défaut dans sa formation. Des études ont montré que la protéine β-amyloïde peut être toxique pour certains types de cellules nerveuses et sa déposition précoce peut être un événement important dans la pathogenèse de la maladie d'Alzheimer.

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In cognitively impaired patients, a definite diagnosis of Alzheimer's disease (AD) can only be established upon neuropathological examination through the quantitation of the characteristic lesions which accompany the extensive neuronal degeneration. These changes consist of intraneuronal neurofibrillary tangles (NFT), extra-cellular amyloid-containing neuritic plaques and cerebrovascular amyloidosis occurring in selected areas only, especially in the hippocampus and the neocortex. It is still not clear what primary event leads to these changes. Furthermore, it is also not known whether accumulation of the lesions themselves results in neuronal dysfunction or whether these are merely a consequence of the initial damage to the cell.

Of all these structures, cerebrovascular amyloid was the first to be characterized, being relatively easily solubilized in guanidinium hydrochloride. Glenner and Wong succeeded in purifying a low molecular weight protein from amyloid-bearing meningeal vessels from individuals with AD[1] and Down's syndrome.[2] The partial amino acid sequence of these small peptides was essentially identical up to residue 28.[3] Although the amyloid core of senile plaques is less easily solubilized, a small peptide of 4-7 kDa was isolated from formic acid-treated cores and shown to be identical to the vascular amyloid protein isolated by Glenner and Wong, but with increased N-terminal heterogeneity.[4]

Screening the brain cDNA libraries with a series of oligonucleotides based on the cerebrovascular amyloid protein sequence led to the identification of a full-length cDNA clone,[5] with an open reading frame coding for a 695-residue protein which has been labelled β–amyloid precursor protein (β-APP). Computer analysis of the β-APP primary structure predicted a transmembrane protein with a large extracellular N-terminal portion and a short cytoplasmic domain. This model placed the C-terminal of the 42-43 amino acid β-amyloid protein within the membrane. Therefore, according to the most simplistic scenario, β-amyloid production, through aberrant degradation of the precursor, would require two cleavages, with one of these occurring within the membrane.

Alternative transcripts of the β-APP gene have been identified. While one species codes for a secreted form of the precursor lacking the transmembrane region (and hence the β-amyloid)[6], the other transcripts, $\beta\text{-APP}_{714}$ and $\beta\text{-APP}_{751}$ have an additional exon of either 19 or 56 amino acids spliced in at residue 289 of $\beta\text{-APP}_{695}$, whereas $\beta\text{-APP}_{770}$ contains both these exons.[7-9] Interestingly, the longer exon codes for a protease inhibitor domain showing 50% homology to the Kunitz family of serine protease inhibitors (KPI).

The mRNA for β-APP has been detected in the nervous system as well as in many tissues outside it (e.g. kidney, heart, muscle).[8,10] The isoform of β-APP containing the KPI domain ($\beta\text{-APP}_{751}$ or $\beta\text{-APP}_{770}$) has been shown to be identical to the protease inhibitor protein nexin II[11] which had previously been purified from the conditioned medium of fibroblasts.[12] This protein nexin II has also been shown to be released from the α-granules of activated platelets[13] and to be an inhibitor of coagulation factor XIa.[14] The function of $\beta\text{-APP}_{695}$ is still unknown; its mRNA is highest in fetal tissue and may be confined predominantly to the nervous system in the adult.[15] On the

From the Departments of Medicine, Neurology & Neurosurgery, McGill Center for Studies in Aging, McGill University, Montreal
Reprint requests to: Dr. Josephine Nalbantoglu, Montreal Neurological Institute, 3801 University Street, Montreal, Quebec, Canada H3A 2B4

other hand, expression of β-$APP_{751/770}$ is higher in peripheral tissues[7-9,15] although it has also been detected in the same neuronal populations as β-APP_{695}.[16,17]

To understand why the metabolism of this family of ubiquitously expressed proteins is altered especially in certain regions of the brain, levels of transcriptional activity have been quantitated by several groups. On Northern analysis, β-APP_{695} seems to be diminished selectively in AD[15,17-19] whereas *in situ* hybridization experiments have shown a selective increase in β-APP_{695}, as compared to β-$APP_{751/770}$, at the level of individual neurons in the nucleus basalis of Meynert and in the locus coeruleus.[16] Northern analysis is not as sensitive and in these cases may rather be reflecting the loss of cells (and hence, total RNA) from these regions. However, there has also been a report of a selective increase of β-$APP_{751/770}$ in hippocampal pyramidal neurons.[20] Despite these somewhat contradictory results, several important points are nevertheless evident: 1) neurons produce β–APP but so do other cell types in the brain (glia, endothelial cells);[21] 2) β-APP is also produced in neurons which are not usually involved in AD such as the Purkinje cells of the cerebellum;[15,21,22] 3) by *in situ* hybidization analysis, some subpopulations of neurons express more β-APP in AD,[16, 20-25] although the level of transcripts is never more than two- to three-fold higher in AD compared to aged-matched controls.

The gene for β–APP spans over 200 kilobases[26] and maps on chromosome 21 near q21.1 - q21.2.[27] This may explain the presence of AD-type lesions in individuals with Down's syndrome as resulting from increased gene dosage; in line with this, the mRNA levels for all β-APP transcripts have been shown to be increased in Down's syndrome fetal brain tissue as compared to normal.[15] To date, no sequence differences have been detected in the few cDNA clones isolated from AD brain tissue.[28,29] Therefore, neither a mutation in the coding sequences, nor a chromosomal rearrangement through gene duplication,[30,31] may be involved in most of the sporadic AD cases. Furthermore, the β-APP gene is not the genetic locus of the Familial Alzheimer Disease (FAD) gene[32,33] which maps, in some early-onset families, at a centromeric location on chromosome 21.[34] However, mutations in the β-APP gene can lead to β-amyloid deposition since in hereditary cerebral hemorrhage with amyloidosis of the Dutch type, a single base pair change in the β-amyloid domain results in cerebrovascular angiopathy and these patients die in their fifties of a cerebral hemorrhage.[35]

With the availability of antibodies against synthetic peptides deduced from the cDNA sequence, approaches are being developed to understand some of the cellular processing of β-APP. In cultured cells, β-APP has been shown to be cleaved very rapidly, with the large N-terminal domain being released to the extracellular medium.[36,37] In cells transfected with the different isoforms of β-APP, constitutive cleavage of the precursor occurs within the β-amyloid region, after amino acid 15;[38,39] presumably, this would preclude accumulation of the β-amyloid peptide. Furthermore, the point mutation detected in hereditary cerebral hemorrhage with amyloidosis of the Dutch type,[35] which results in a Glu to Gln alteration at position 22 of the β-amyloid domain (5 residues from the constitutive cleavage site), would be expected to alter proteolytic specificity through the modification of ionic charges in the vicinity of the cleavage site.

Selkoe et al.[40] have identified the native β-APP in post-mortem brain and nonneural human tissues as 110 to 135 kDa proteins present in various membrane-rich subcellular fractions. The transmembrane portion was shown to be ~11 kDa after reaction with an anti-C-terminal antibody.[40] It is not clear from these studies what proportion (if any) of the intact (non-cleaved) precursor remains on the plasma membrane. Some of these different species of proteins were also detected in the cerebrospinal fluid[36,41] and plasma.[42] However, to date, no form of β-APP containing an intact β-amyloid domain has been detected either in plasma or cerebrospinal fluid. This would suggest that there is no appreciable pool of circulating intact precursor protein which could serve as the starting material for the amyloid deposits.

The studies on the processing of β-APP suggest that abnormal deposition of β-amyloid involves several steps: 1) absence of normal cleavage; 2) induction of cleavage in the extracellular domain at a site 15 amino acids away from the normal one, as well as 3) unmasking of a cleavage site usually buried in the cell membrane. If the abnormal production of β-amyloid is due to aberrant intracellular trafficking and processing of the newly synthesized precursor, it is not clear how β-amyloid ends up accumulating extracellularly. On the other hand, if the intact precursor is already inserted into the cell membrane prior to the aberrant cleavage, it is difficult to envisage how the site situated within the membrane becomes exposed to proteolytic activity. Down's syndrome and the Dutch-type cerebral hemorrhage represent two examples in which β-amyloid might accumulate either through overexpression or mutation of the β-APP protein. Although, in both cases, the normal proteolytic process is subverted, extensive deposits of β-amyloid do not occur elsewhere in the brain or in peripheral tissue, as would be expected. Thus, it must be assumed that either constitutive cleavage of the precursor still occurs or mechanisms exist for degrading the β–amyloid as it is formed. In AD, no mutation in β-APP has been identified, nor has consistent overexpression been detected in all involved neuronal populations. The metabolic perturbation may be occurring at the level of the proteolytic mechanisms.

Great progress is being made in elucidating the nature of the β-amyloid deposits. By immunocytochemical methods it has been shown that amorphous plaques, labelled "preamyloid", can be detected in brain tissue as early as 40 years of age in a normal population,[43] and 13 years of age in individuals with Down's syndrome.[44] Furthermore, the rate of deposition is similar in both groups, with the process beginning 40-50 years earlier in Down's syndrome.[44] The studies in Down's syndrome have been confirmed by other workers[45,46] and the conclusions remain the same: the diffuse, amorphous "preamyloid" precedes senile plaques, does not stain with Congo red or thioflavin, nor does it contain degenerating neurites. Although it cannot be concluded that in individuals who, at death, were free of cognitive changes, the "preamyloid" deposits detected would have subsequently developed into senile plaques with amyloid cores, all patients with Down's syndrome develop the typical AD neuropathology if they survive beyond the age of 40. Thus, the observation of "preamyloid" in younger Down's syndrome individuals would suggest that deposition of β-amyloid is an early event and not merely the consequence of extensive neuronal degeneration. In heredity cerebral hemorrhage with amyloidosis of Dutch origin, "preamyloid" is present in the cortex although

no senile plaques have been detected and dementia has not been reported.[47] Death of these patients of cerebral hemorrhage in their early fifties may preclude development of the characteristic neuropathology and dementia.

At the biochemical level, the presence of "preamyloid" may have important effects on surrounding cells. The β-amyloid protein has recently been shown to be toxic to primary rat hippocampal cultures.[48] When 4-day-old cultures were treated with β-amyloid at a concentration of 20 x 10^{-6} M, a 50% decrease was observed in the number of viable pyramidal neurons. The effective concentration of β-amyloid could be decreased to 1 x 10^{-12} M when the cultures were co-incubated with NGF. Similarly, PC12 cells transfected with a β-amyloid/C-terminal construct were shown to degenerate in the presence of NGF without extending neurites and entering their differentiation program.[49] In this study, the neurotoxic activity could be found in the conditioned medium of transfected cells (either PC12 or NIH 3T3) and could be partially blocked by antibodies against the β-amyloid. At first view, the mechanism of this neurotoxicity is unclear since the protein produced by this construct does not contain a signal peptide and the hydrophobic molecule would be expected to accumulate intracellularly without ever being secreted. Nevertheless, these studies show that β-amyloid may be toxic to certain neuronal cell types and its early deposition may be an important element in the pathogenesis of AD.

Note added in proof

Goate et al (Nature 1991; 349: 704-706) have shown that in two unrelated families a missense mutation close to the C-terminus of the β-amyloid protein segregates with AD.

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