Proteolytic processing of the amyloid-β protein precursor of Alzheimer’s disease

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Abstract

The proteolytic processing of the amyloid-β protein precursor plays a key role in the development of Alzheimer’s disease. Cleavage of the amyloid-β protein precursor may occur via two pathways, both of which involve the action of proteases called secretases. One pathway, involving β- and γ-secretase, liberates amyloid-β protein, a protein associated with the neurodegeneration seen in Alzheimer’s disease. The alternative pathway, involving α-secretase, precludes amyloid-β protein formation. In this review, we describe the progress that has been made in identifying the secretases and their potential as therapeutic targets in the treatment or prevention of Alzheimer’s disease.

Introduction

Alzheimer’s disease (AD) is the most prevalent form of dementia in the elderly, causing progressive memory loss, confusion and behavioural changes. The presence of extracellular amyloid deposits in the brain is a key pathological feature of AD. Amyloid occurs in the neuropil in the form of amyloid plaques and around cerebral blood vessels in the form of cerebral amyloid angiopathy [1]. The major protein component of amyloid in the AD

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brain is the amyloid-β protein (Aβ) [2], a 4 kDa polypeptide that is derived from a much larger precursor known as the amyloid-β protein precursor (APP) [3] (Figure 1). According to the amyloid hypothesis, the accumulation of Aβ in the brain is an important step in the pathogenesis of AD.

In this review, we describe our current understanding of the proteases involved in the proteolytic processing of APP. Inhibitors or activators of these proteases are emerging as key targets for the treatment of AD. The identification of an effective therapeutic strategy would be of enormous benefit as AD is one of the most common diseases associated with aging.

**APP: structure and processing**

The APP gene is found on chromosome 21 and encodes a type 1 transmembrane glycoprotein [3]. The gene contains 18 exons, and parts of exons 16 and 17 encode the Aβ region, which lies partially within the ectodomain and partially within the transmembrane domain of APP. Alternative splicing of the APP mRNA can yield a number of isoforms. All forms of APP consist of a large N-terminal ectodomain that contains a signal peptide, a cysteine-rich domain and an acidic region, a short transmembrane domain, and a C-terminal cytoplasmic tail (Figure 1). Some isoforms also contain a domain that shows identity with Kunitz-type protease inhibitors and

![Figure 1. Functional domains of APP and the amino-acid sequence of the Aβ region](image)

APP has a large ectodomain, with a signal peptide (SP), a cysteine-rich region, an acidic domain, a region with high identity with the Kunitz-type protease inhibitors (KPI) and a domain sharing identity with the OX-2 protein (OX2). The Aβ sequence lies partially within the ectodomain and partially within the transmembrane domain (TM). The cleavage sites for α-, β- and γ-secretases are shown, as are the mutations in APP that are known to cause FAD.
a region that shares identity with the OX-2 protein. In neurons, the major isoform contains 695 amino acid residues (APP695), and lacks the Kunitz-type protease inhibitor and OX-2 domains [4].

There are at least two pathways by which APP is proteolytically processed (reviewed in [5]). This processing results from the action of proteases that are termed ‘secretases’ (Figure 2). A major pathway involves cleavage by α-secretase, which cuts the Aβ sequence on the C-terminal side of Lys\(^{16}\). This cleavage destroys the intact Aβ sequence. Alternatively, APP can be processed by β-secretase at the N-terminus of the Aβ sequence, producing a 99-amino-acid-residue C-terminal fragment, known as C99. Further processing of C99 by γ-secretase within the transmembrane region releases Aβ, which is approx. 40 amino-acid residues in length. Two major forms of Aβ are produced. Approx. 90% of the Aβ secreted contains 40 amino-acid residues (Aβ\(^{40}\)), and a minor form, comprising approx. 10% of the total Aβ, contains 42 (Aβ\(^{42}\)) or 43 (Aβ\(^{43}\)) amino acids and is referred to as ‘long Aβ’.

It is now generally believed that the accumulation of long Aβ in the brain of AD patients is intimately associated with neurodegeneration. Cell culture studies show that Aβ is toxic to neurons in culture and that the aggregation of the polypeptide into higher molecular mass oligomeric species is necessary for

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**Figure 2. Non-amyloidogenic and amyloidogenic pathways of APP processing**

Cleavage of APP by α-secretase produces sAPP\(_{\alpha}\), which is secreted, and a C-terminal fragment C83. C83 is further processed by γ-secretase to release p3. The amyloidogenic pathway involves cleavage of APP by β-secretase to produce sAPP\(_{\beta}\) and C99, which is subsequently cleaved by γ-secretase to release Aβ. Aβ is secreted, and can form aggregates which have neurotoxic properties.
this effect [6]. As long Aβ aggregates more readily than Aβ40, these aggregates may contribute significantly to Aβ toxicity and amyloid plaque formation, by seeding the aggregation of the more abundant Aβ40.

**APP mutations**

The strongest evidence that the production of Aβ is linked to the pathogenesis of AD comes from the identification of familial AD (FAD) mutations within the APP gene. FAD is inherited in an autosomal dominant manner, with the onset of clinical features usually occurring before the age of 65 years. In general, the phenotype and neuropathology of FAD is indistinguishable from late-onset ‘sporadic’ cases of the disease. This strongly suggests that the aetiologies of FAD and late-onset AD are similar.

A number of mutations in the APP gene have been identified which can cause early-onset FAD [4]. These mutations are clustered around the region that encodes Aβ (Figure 1). In general, all of these mutations have been found to increase the production of long Aβ (or an N-terminally truncated form of long Aβ). A double mutation located at codons 670 and 671 on the N-terminal side of the β-secretase cleavage site was first identified in a Swedish family. Another mutation at codon 692 near the α-secretase site was identified in a Flemish family. Several FAD mutations (London, Florida, French, Australian and Austrian) are clustered around the γ-secretase site within the transmembrane domain. These mutations change the site of γ-secretase cleavage to increase the proportion of long Aβ that terminates at amino acid residue 42 [7].

A reasonable strategy for AD therapy would be to inhibit Aβ toxicity, but the mechanism by which Aβ causes neurotoxicity is unclear [8]. Another strategy is to increase clearance of Aβ from the brain, and recent studies [9] suggest that a vaccination approach may be effective. However, at the time of writing, the safety of this approach and its efficacy are unknown. Considerable effort is now being expended on the design of compounds which inhibit Aβ production by acting on the β- and γ-secretases. In this review, we summarize our current knowledge about the α-, β- and γ-secretases and the compounds that inhibit or activate them.

**α-Secretase**

α-Secretase provides a major route for APP processing, by cleaving on the C-terminal side of Lys16 of the Aβ sequence (Figure 2). This cleavage produces a soluble N-terminal fragment (sAPPα) and a membrane-bound C-terminal fragment (C83). C83 undergoes further processing by γ-secretase, releasing a fragment known as p3.

α-Secretase has both a basal (constitutive) activity and a regulated activity, which is stimulated by protein kinase C (PKC) [4]. The activation of PKC via phorbol esters or via M1 or M3 muscarinic receptors increases α-secretase-mediated cleavage. PKC inhibitors reduce the activity of α-secretase to a residual
level (the basal activity) [10]. The stimulation of the α-secretase pathway does not always cause a corresponding decrease in Aβ production, indicating that APP processing may vary between cell types.

Tumour necrosis factor-α (TNF-α)-converting enzyme (TACE/ADAM17) is one of the candidate α-secretases. Initially identified as the protease responsible for pro-TNF-α cleavage, TACE’s action of releasing TNF-α from its membrane-bound precursor is very similar to the processing of APP by α-secretase [11]. Buxbaum et al. [11] showed that knock-out of the TACE gene in primary embryonic fibroblasts almost completely abolished PKC-stimulated sAPPα production, but had little effect on basal sAPPα secretion. Furthermore, TACE was found to cleave a short synthetic peptide at the α-secretase site, indicating that the enzyme has the appropriate cleavage specificity [11].

TACE belongs to the ADAM (a disintegrin and metalloprotease) family, which is structurally related to the PIII class of snake venom metalloproteases (see Chapter 11). Members of this family contain a propeptide domain that must be removed to enable the enzyme to become active, a metalloprotease domain (which contains the catalytic activity), a disintegrin domain, a cysteine-rich domain and a transmembrane domain (Figure 3).

Owing to its structural similarity to TACE (Figure 3), ADAM10, another member of the ADAM family, has also been proposed to be an α-secretase. ADAM10 can cleave APP at the α-secretase site, and overexpression of ADAM10 increases sAPPα levels, which can be further increased by PKC [12]. In contrast to TACE, ADAM10 has both the basal and regulated components of α-secretase activity. A dominant-negative form of the protein inhibits both basal and phorbol ester stimulated α-secretase activity [12]. Furthermore, ADAM10 mRNA, unlike TACE, is co-localized with APP and a putative β-secretase candidate in the mouse brain [13].

A third putative α-secretase, PC7, belongs to a different family of proteases known as the proprotein convertases (PCs). This family of proteases is involved in proprotein processing (see Chapter 7). All members of this family are serine proteases that have a catalytic triad of residues at the active site (Figure 3). PC7 is expressed in a range of tissues and is localized to the trans-Golgi network. While overexpression of PC7 increases sAPPα production [10], it is still unclear whether PC7 is an authentic α-secretase. Recent evidence suggests that PC7 may proteolytically cleave the inactive pro-ADAM10 into the active form [14].

**β-Secretase**

β-Secretase-mediated cleavage is the penultimate step in the production of Aβ. β-Secretase cleaves at two sites within the ectodomain of APP. The main cleavage site is located on the N-terminal side of Asp1 of the Aβ sequence, with a minor cleavage site at Glu11 (Figure 1). Cleavage at Asp1 produces a
soluble C-terminally truncated fragment of APP that is eventually secreted (sAPPβ), and a membrane-bound fragment (C99). Subsequent cleavage of C99 by γ-secretase releases Aβ, which is also secreted (Figure 2). Most of the β-secretase appears to be localized in the Golgi apparatus [15] and astrocytes possess a low level of β-secretase activity. Thus, neurons are likely to be the major cell type contributing to Aβ production in the central nervous system.

In late 1999, four groups almost simultaneously reported the discovery of a protein that fulfilled all of the requirements of β-secretase [15–18]. This protease is known as BACE1 (β-site APP-cleaving enzyme 1), but has also been referred to as Asp2 and memapsin 2. BACE1 represents the first member of a new family of aspartic proteases. The protease is expressed in low levels in the periphery, but has a high expression level in the brain, particularly in neurons in the hippocampus, cortex and cerebellum [15,17], coinciding with the expected distribution of β-secretase. The gene encoding BACE1 is located on chromosome 11 and has an open reading frame of 501 amino acids. The gene encodes a
type 1 integral membrane protein with several distinctive domains, including a catalytic domain, which contains two active-site regions containing the signature sequence of an aspartic protease (DT/SGT/S) (Figure 3). Consistent with its membership of this protease family, mutating the aspartate residues within the active site renders the protein inactive [15,16]. Despite conservation within the active-site region, BACE1 only has 30–37% identity with other known aspartic proteases. BACE1 is the first reported aspartic protease that contains a transmembrane domain, and also the first aspartic protease known to be insensitive to the protease inhibitor pepstatin.

Several lines of evidence demonstrate that BACE1 is a β-secretase. BACE1 can cleave full-length APP at both the major (Asp1) and minor (Glu11) β-secretase sites. The Swedish APP mutation, which increases β-secretase activity in cell culture, also increases the BACE1-catalysed cleavage at position 1 of the Aβ sequence. In cells that overexpress BACE1, there is increased production of all β-secretase products (Aβ, sAPPβ and C99) [15–17]. Furthermore, BACE1 antisense oligonucleotides inhibit β-secretase activity [15]. There is almost total loss of Aβ compared to controls in BACE1 knock-out mice [19], and cortical neurons cultured from BACE1 knockout embryos do not produce any β-secretase-derived fragments of APP (C99, Aβ1–40/42 and Aβ11–40/42), although there is an increase in α-secretase-generated products [20]. Taken together, these findings strongly argue that BACE1 is the major β-secretase in neurons.

BACE1 undergoes several post-translational modifications. In the Golgi apparatus, phosphorylation of the C-terminus occurs and the pro-peptide domain is cleaved, producing the mature form of BACE1. A proprotein convertase (furin) cleaves off the propeptide domain of BACE1. Unlike most aspartic proteases, BACE1 does not appear to require propeptide cleavage to become active; pro-BACE1 does not significantly differ in activity from mature BACE1.

Another transmembrane aspartic protease has been named BACE2 owing to its high identity with BACE1 (Figure 3). Although BACE2 can cleave APP at the β-secretase sites (Asp1 and Glu11), it is more efficient at cleaving Aβ internally at Phe19 and Phe20 [21]. Furthermore, BACE2 is found primarily in the periphery, with negligible levels in the brain [21]. Therefore, although BACE2 may have some β-secretase ability, it is unlikely (owing to its cleavage sites and localization) to contribute significantly to Aβ formation in the brain. Nonetheless, selective inhibitors of BACE1, which do not block BACE2, may need to be identified if the inhibition of BACE2 has toxic consequences.

**γ-Secretase**

Cleavage of the β-secretase product C99 by γ-secretase is the final step in the production of Aβ. γ-Secretase can cut C99 at a number of sites (Figure 1),
producing a variety of Aβ species with different C-termini. Aβ40 and Aβ42 (long Aβ) are major products of γ-secretase activity.

The location of the γ-secretase cleavage site is unusual as it resides within the transmembrane region. Several transmembrane proteins are known to be cleaved within or close to their membrane-spanning domain. This phenomenon, known as regulated intramembrane proteolysis, is discussed in more detail elsewhere in this volume (Chapter 12). At present, the mechanism by which regulated intramembrane proteolysis occurs is unknown. Cleavage by proteases is a hydrolytic event that requires the presence of water. It is unclear how water can get to the active site in the hydrophobic environment of the membrane. Cleavage may occur within the membrane by some as yet undefined mechanism. Alternatively, proteolytic cleavage may occur after the polypeptide has been removed from the lipid bilayer.

Although a number of proteins have been proposed to have γ-secretase activity, thus far, none has convincingly demonstrated the characteristics required for γ-secretase. Integral membrane proteins known as presenilins are γ-secretase candidates. The genes for presenilin-1 (PS1) and presenilin-2 (PS2) are located on chromosomes 14 and 1 respectively, and encode large proteins with 67% identity [22]. The proposed structure of the presenilins has eight transmembrane regions with a large cytosolic loop (Figure 4). The presenilins can be cleaved by a protease known as presenilinase, producing N- and C-terminal fragments of 35 and 20 kDa respectively, as well as by caspase at an adjacent site in the loop. The fragments generated by presenilinase remain associated after cleavage.

Mutations in the presenilin genes can lead to early-onset AD, and are responsible for more familial cases of AD than mutations of the APP gene. At the time of writing, 85 mutations in the PS1 gene have been identified and five are known within the PS2 gene [22]. The close association between presenilin and γ-secretase is suggested by the demonstration that knock-out of the presenilin genes results in knock-out of γ-secretase activity. The absence of presenilins eliminates Aβ production, with accumulation of α- and β-secretase-cleaved products. Furthermore, mutations in presenilins affect the γ-secretase cleavage site, increasing the amount of Aβ42 produced. Two aspartate residues, located in the sixth and seventh transmembrane domains, that are critical for presenilin function are predicted to align in the membrane and have been proposed to give rise to a catalytic cleavage similar to that of the aspartic proteases [22].

However, the relationship between presenilins and γ-secretase remains unclear. Overexpression of presenilin does not lead to increased γ-secretase activity, nor has either of the presenilins been shown to possess protease activity. Furthermore, the subcellular localization of PS1 does not always appear to correspond with the localization of γ-secretase activity [23].

Although presenilins may not be identical with γ-secretase, they may form part of a high molecular mass complex that contains the γ-secretase. Immunoprecipitation of presenilins leads to the co-immunoprecipitation of
secretase activity. Furthermore, photoactivatable γ-secretase inhibitors bind to presenilin heterodimers [24]. Recently, a type 1 transmembrane protein known as nicastrin, which binds to full-length APP as well as α-secretase- and β-secretase-cleaved APP, and forms a high molecular mass complex containing presenilin, was identified [25]. The identification of other proteins in this complex is an active area of investigation at present.

Secretases as therapeutic targets for AD

As the proteolytic processing of APP to Aβ is central to the pathogenesis of the disease, drugs that inhibit amyloidogenic processing of APP are potential therapeutic agents. For example, the upregulation of α-secretase activity may channel APP processing down a non-amyloidogenic pathway, thereby decreasing Aβ production. A number of agents increase α-secretase activity activating PKC, e.g. muscarinic receptor stimulation [5]. Therefore, cholinesterase inhibitors that boost levels of acetylcholine may increase non-amyloidogenic processing of APP, by decreasing Aβ production and slowing the progression of AD. However, studies using long-term anti-cholinesterase treatment of AD patients have provided little clear evidence of a definitive slowing of the progression of the disease. Similarly, there is as yet no evidence that muscarinic receptor agonists improve cognition in AD.

At present, the most attractive therapeutic strategy is based upon the direct inhibition of the amyloidogenic pathway. One approach is to inhibit β-secretase. The crystal structure of BACE1 has been determined recently, and indicates that the active site is quite distinctive, being more hydrophobic and open than that of...
other aspartic proteases [26]. This is an advantage for selective inhibitor design. Several compounds that inhibit Aβ production by reducing cleavage by β-secretase have been identified. One of the most potent of these is a tripeptide aldehyde that contains reduced amide isosteres that have been used previously for aspartic protease inhibition. As BACE1 gene knock-out mice are viable, with no major physical or cognitive deficits [19], this suggests that inhibition of BACE1 may be an effective therapeutic strategy with minimal toxic consequences.

The alternative approach is to inhibit γ-secretase. This should also decrease Aβ production. A number of effective γ-secretase inhibitors have been reported; one very potent inhibitor is a compound with an hydroxyethylene dipeptide isoster, which acts as a transition state analogue mimic at the catalytic site of an aspartic protease [27]. However, the efficacy of γ-secretase inhibitors for AD therapy is uncertain, as there may be other normal physiological substrates for γ-secretase. For example, γ-secretase can also cleave a protein called Notch, which is similar to APP, and is essential for a number of cellular functions, such as cell fate and differentiation [28]. The intramembranous cleavage of Notch releases the intracellular domain, which translocates to the nucleus and activates the CSL family of transcription factors. Notch function relies on this cleavage; its absence causes a lethal phenotype in embryos. The protease required for Notch processing is strikingly similar to γ-secretase (see Chapter 12), and the majority of inhibitors are unable to distinguish between the two proteolytic activities. However, the recent development of γ-secretase inhibitors that have greater selectivity for APP processing than for Notch processing [29] indicates that, despite their similarity, the γ-secretase-like proteases cleaving APP and Notch may be pharmacologically different.

Conclusions

There is very good evidence to suggest that the accumulation of Aβ is the underlying cause of AD, and that inhibition of Aβ production may prevent the progression of the disease. Much progress has been made in understanding the proteases responsible for the cleavage of Aβ from APP. α-Secretase, the protease responsible for the majority of APP processing, may be associated with one or more members of the ADAM family of metalloproteases. β-Secretase has been identified as an aspartic protease (BACE1) and γ-secretase has yet to be identified definitively. At the time of writing, several secretase inhibitors are being tested in phase 1 clinical trials. The results of these human trials will eventually indicate whether secretase inhibitors are effective in the treatment or prevention of AD, but they may also provide the first direct proof of the amyloid hypothesis of AD.
Summary

- The accumulation of Aβ is believed to be the underlying cause of the neurodegeneration seen in AD patients. This is known as the amyloid hypothesis.
- Aβ is formed by the proteolytic cleavage of its precursor, APP.
- Cleavage of APP by β- and γ-secretases produces the N- and C-termini of Aβ respectively. Cleavage by α-secretase destroys the Aβ sequence.
- A number of α-secretase candidates have been proposed, and it appears likely that more than one protease is responsible for cleavage at the α-secretase site.
- A recently identified protease named BACE1 is responsible for the majority of β-secretase activity in the central nervous system.
- There are a number of sites at which γ-secretase can cleave, all of which are located in the transmembrane domain of APP. Although the enzyme(s) responsible for these cleavages has not been identified, the presenilins are likely to play a major role.
- The inhibition of Aβ production is an attractive therapeutic strategy. This may be achieved by the selective inhibition of β-secretase and/or γ-secretase.

References


