Memapsin 2 (\(\beta\)-secretase) as a therapeutic target

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Abstract

As \(\beta\)-secretase, memapsin 2 cleaves amyloid-\(\beta\) precursor protein, which leads ultimately to the onset of Alzheimer’s disease. As such, memapsin 2 is an excellent target of inhibitor drugs for the treatment of this disease. Here we describe the tools for memapsin 2 inhibitor design that have been developed and results from the structure-based inhibitor design. Strategy for the design of memapsin 2 inhibitors with pharmaceutical potential is also discussed.

Introduction

Accumulation of the amyloid-\(\beta\) peptide (A\(\beta\)) in the brain is now believed to be the primary cause of the neurodegeneration and progression of Alzheimer’s disease (AD) [1]. A\(\beta\) is a fragment of amyloid-\(\beta\) precursor protein (APP), released by two proteases known as the \(\beta\)- and \(\gamma\)-secretes. Since there is no effective drug for treatment of AD at present, there is an intense interest in potential therapeutic targets. \(\beta\)-Secretase is a particularly exciting therapeutic target for several reasons. This protease initiates the first step in A\(\beta\) production. Thus, inhibition of its activity would block the entire cascade of AD pathogenesis. Additionally, the fact that \(\beta\)-secretase is an aspartic protease has also raised the hope that its therapeutic inhibitor can be as successful as that against HIV protease. Finally, the absence of a functional \(\beta\)-secretase gene in mice did not produce a clear phenotype, suggesting that the inhibition of this protease in therapy is physiologically tolerable [2–4].

\(\beta\)-Secretase is a membrane-anchored aspartic protease [5–9]. Since this historical name did not appropriately reflect its protease nature, several new names were given. The name memapsin 2 [9] is rooted in ‘membrane aspartic protease of the pepsin family’ and the numeral 2 distinguishes it from a homologous protease, memapsin 1. This name conforms with the nomenclature of aspartic proteases recommended by the IUBMB’s Enzyme Nomenclature ([10]; also at http://www.chem.qmw.ac.uk/iubmb/enzyme/EC34/3421.52.html#23), e.g. pepsin, gastric, cathepsin, etc., and thus will be used here. Other names used were BACE [5], ASP-2 [6,8] and membrane-bound aspartic protease [7].

Memapsin 2 activity and first-generation inhibitors

As has been observed with other mammalian aspartic proteases, memapsin 2 mRNA encodes for a pre-pro-memapsin 2. Uniquely present is the C-terminal extension of a transmembrane and intracellular region. Cellular processing by furin at an Arg\(^{45}\)-Glu\(^{46}\) bond in the pro region produces Glu\(^{46}\)-memapsin 2 [11–14]. The optimal pH for memapsin 2 lies in the range 3.5–4.5 [5,9,15]. Recombinant pro-memapsin 2 is proteolytically active [5,10] due to a transient blocking of the active site by the pro-peptide [15]; thus, the differences in activity of different species of pro-memapsin 2 and memapsin 2 are found in \(k_{\text{cat}}\), but not in \(K_{\text{m}}\). The \(K_{i}\) values determined using pro-memapsin 2 are, however, several-fold higher than those obtained from mature memapsin 2 processed by proteases such as clostripain [15].

Pepstatin A, a general inhibitor for aspartic proteases, inhibits memapsin 2 poorly with an \(IC_{50}\) of 50 mM [9]. Pepstatin inhibitors based on statine, a constituent amino acid of pepstatin A, was able to achieve moderate to good inhibition potency [17]. Based on early specificity information, we designed transition-state inhibitor OM99-2 (Glu-Val-Asn-Phe-Ala-Glu-Phe-Phe; where \(\Psi\) represents a hydroxyethylene transition-state isostere) [7] with a \(K_{i}\) against memapsin 2 of 1.7 nM and a \(K_{i}\) of 1.0 nM [15]. These results from the first-generation inhibitors designed from memapsin 2 specificity demonstrated good promise that high a potency of inhibition can be achieved.
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Figure 1
X-ray crystal structure of the active site of memapsin 2 complexed with inhibitor OM99-2

Blue nitrogen and red oxygen atoms of inhibitor (brown), which hydrogen-bond to atoms of memapsin 2 residues (blue), are linked by broken lines. Positions of substrate are labelled Pn.

Crystal structure of memapsin 2 complexed to inhibitor OM99-2

In order to learn the interactions of the memapsin 2 active site with an inhibitor, we solved the X-ray crystallographic structure of recombinant memapsin 2 protease domain bound to inhibitor OM99-2 [18]. The overall folding of the memapsin 2 catalytic domain is homologous to other mammalian aspartic proteases [18]. Most of the C-terminal extension unique for memapsins is part of the folding of the globular catalytic unit. The inhibitor is bound in the substrate-binding cleft located between the N- and C-terminal lobes. Six of the eight OM99-2 residues (P4-P4) are bound in the active site of memapsin 2 in an extended structure and their respective binding sites (S4-S4) are well defined from atomic contacts with the inhibitor side chains (Figure 1). The P4 and P4 side chains have less interaction with the enzyme and are mobile in the crystal structure. There is extensive hydrogen bonding between memapsin 2 to the polypeptide backbone of OM99-2 and some of the more polar inhibitor side chains, such as P1-Glu and P2-Asn. On the other hand, the contacts between the protease subsites to side chains at P3-Val, P1-Leu, P1-Ala and P1-Ala are mostly hydrophobic and van-der-Waal interactions. This structural model is important for the design of a new generation of memapsin 2 inhibitors.

Structure-based design of second-generation inhibitors

The crystal structure of memapsin 2-OM99-2 was used to design smaller yet still potent inhibitors. In the X-ray crystal structure, the inhibitor chain turns at the P4 position and the P4-P4 side-chain residues are not involved in any specific interaction in this region. In the design of new inhibitors [19], we substituted the P4-P4 residues with a simple benzylamine group and installed different side-chain structures at P3, P4, P4 and P4. The P4 residue was also simplified or eliminated. The most potent resulting inhibitor, GT-1017 (Figure 2), was 722 Da with a Ki of 2.5 nM [19]. Compared with OM99-2, which is 1100 Da with a Ki,
of 1.7 nM, the structure-based design reduced the inhibitor size considerably yet retained the potency. These results show excellent promise for the structure-based design of memapsin 2 inhibitors in the future.

**Complete subsite specificity of memapsin 2**

Although OM99-2 had excellent inhibition potency, it was designed based on limited information about memapsin 2 specificity. Like other mammalian aspartic proteases, the active site of memapsin 2 can accommodate eight subsites, six of which (P₂–P₆) have clear interaction with the enzyme [18]. We have since determined the complete residue preferences in all eight memapsin 2 subsites [20] (Figure 3). A compiled sequence for the most preferred residue in each subsite is EIDL/MVLD (where / denotes a scissile bond). Second- and third-ranking preferred residues in each subsite are QVNF/EIWE and DLMM/QAVW, respectively. It is clear that memapsin 2 has non-stringent specificity in all eight subsites. This is particularly true for subsites P₃ and P₄', in agreement with the lack of side-chain interaction of these two inhibitor residues in the crystal structure. It is also interesting to note that in the sequence around the β-secretase site of Swedish APP, residues in P₁–P₁ (EVNL) are among the most or second-most preferred, while residues in P₁'–P₄' (DAEF) are among the most favoured. The most dramatic gain in the hydrolytic efficiency due to APP Swedish mutation appears to be the Lys-to-Asn substitution at P₂. Since the P₁ Met-to-Leu substitution has a lesser effect, this raises an interesting question of whether an APP P₁ mutation alone to residues such as Asn or Asp is sufficient to bring about an early onset of AD.

**Figure 3**

Subsite specificity of memapsin 2

An increased value for relative kcat/Km in each panel indicates a preference for that amino acid present at the indicated (boxed) position of the substrate EVNL-AAEF [20], where the dash represents the cleavage site.
Subsite residue preference has also been determined for memapsin 2 inhibitors using a combinatorial peptide-inhibitor library with residues randomized at four subsite positions [20]. The consensus residues (P$_3$, L or I; P$_2$, D; P'$_2$, V; P'$_3$, E) are in good agreement with substrate specificity information (Figure 1), except at the P'$_3$ position.

**Strategy in the design of new-generation inhibitors**

The design of memapsin 2 inhibitor drugs is more challenging than most because, in addition to the desired properties, such as high potency, high selectivity, low toxicity and good pharmacokinetic properties, they must also penetrate the blood–brain barrier to reach the brain. Compounds that freely penetrate the blood–brain barrier are below 500 Da. Whether having both the desired properties and this size range can be attained in memapsin 2 inhibitors is uncertain. It is likely, however, that inhibitors with pharmaceutical potential in the size range of 600–700 Da can be designed successfully. Some inhibitors in this size range may reach the brain. This contention is supported by the precedent that an HIV protease inhibitor, indinavir (613 Da), penetrates the blood–brain barrier [21]. We have reduced the inhibitor size from 1100 Da for OM99-2 to 722 Da for GT-1017 without significantly compromising the inhibition potency. Such results suggest that inhibitors with desired properties in the size range 600–700 Da are attainable.

Based on the subsite preference of memapsin 2, we have designed a new inhibitor, OM00-3 (Glu-Leu-Asp-Leu$_	ext{P}$_Ala-Val-Glu-Phe), which is three times more potent than OM99-2 [20]. A new crystal structure of OM00-3 complexed to memapsin 2 revealed interactions of P$_3$ and P'$_3$ residues with the protease not observed in the OM99-2–memapsin 2 structure [18]. This structure provides a new option in the design of memapsin 2 inhibitors in which P$_3$ and P'$_3$ are included, while the residues at the N-terminal side of the inhibitors may be omitted to reduce the size. Our experience supports the view that the structure-based inhibitor-design cycle would be of great value in the evolution of clinically useful memapsin 2 inhibitors.

One of the most challenging problems in the design of memapsin 2 inhibitors may be the inhibition selectivity against memapsin 1. The specificity of the two memapsins is very similar [20] and some of the potent memapsin 2 inhibitors inhibit memapsin 1 equally well [19]. A structure of memapsin 1’s interaction with an inhibitor such as OM000-3 would be very helpful for future selectivity design.

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**References**


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Abstract
Development of Alzheimer’s disease (AD) pathology appears to be causally related to age-dependent changes in the metabolism of the amyloid-β peptide (Aβ), leading to its enhanced aggregation and deposition. γ-Secretase is a crucial enzyme for the generation of Aβ from the amyloid-β precursor protein and thus represents a valid potential therapeutic target for the treatment or prevention of AD. Enzyme activity has been shown to be dependent on the expression of presenilins and the identification of inhibitors containing transition-state analogue mimics, together with mutagenesis and knockout studies, confirms that presenilins may provide at least a component of the catalytic site for this putative aspartyl protease. Considerable effort has been expended to identify compounds which specifically reduce γ-secretase activity in the central nervous system, and those with the appropriate properties are being utilized in on-going proof-of-concept studies in animals and humans, to determine the extent and duration of γ-secretase inhibition required to elicit therapeutic benefits.

γ-Secretase-mediated substrate cleavage appears to fall into the category of ‘regulated intramembrane proteolysis’. By virtue of its mechanistic similarities, the effects of γ-secretase inhibitors on proteolysis and signalling through other substrates, such as Notch, has to be determined carefully, since this is likely to impact on the clinically safe dose of these compounds.

Proteolytic processing of the amyloid-β precursor protein (βAPP) [1] by sequential action of β-secretase and γ-secretase enzymes releases the amyloid-β peptide (Aβ) [2]. According to the amyloid cascade hypothesis [3] this peptide entity is thought to be the cause of Alzheimer’s disease (AD). Therefore, inhibiting either of the two critical enzymes involved in Aβ peptide generation provides opportunities to develop drugs which could have the potential to slow down the progression and delay the onset of AD. β-Secretase (β-site APP-cleaving enzyme (BACE1)) has been cloned [4–8] and was shown to be a transmembrane aspartyl protease cleaving βAPP at Asp-11 and Glu-11 of the Aβ sequence. A second homologue, BACE2, was identified [8–10] that is capable of cleaving at Asp-1 and more efficiently at Phe-19 or Phe-20 of the Aβ sequence [11]. This enzyme, however, appears to play a minor role in the generation of Aβ, as BACE1 deficiency is sufficient to abolish Aβ generation in neurons and brains of knockout animals [12]. Whereas BACE has been characterized intensively using over-expressing cell lines [13] and purified recombinant enzyme [14], the critical enzyme releasing Aβ from membrane-bound βAPP-processing intermediates, γ-secretase, has been much more elusive. This enzyme appears to have an extremely loose substrate specificity [15] and cleaves rather at a specific position relative to the membrane bilayer [16]. γ-Secretase itself displays characteristics of an aspartyl protease as it is inhibited by structurally diverse transition-state mimetics for this class of protease. These include compounds with moderate affinities such as substrate-based difluoroketones [17] or an inhibitor with potency in

Key words: Alzheimer’s disease, amyloid-β peptide, presenilin.
Abbreviations used: AD, Alzheimer’s disease; Aβ, amyloid-β peptide; PS1/PS2, presenilins 1 and 2; βAPP, amyloid-β precursor protein; BACE, β-site APP-cleaving enzyme; NICD, Notch intracellular domain.

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