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Course Code : SXM390
Assignment : Project

The mechanisms of binding of $BACE_1$ to the amyloid precursor protein, its structure & its inhibition.

By

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1 Abstract

Alzheimer's disease (AD) is complicated in its pathogenesis in that at least two radically differing ideas pervade as to its origin. The two leading pathogenetic hypothesis are completely different in their explanations and focus. However, and at present, the most popular agent for the propagation of AD is believed to be by means of the accumulation of the β amyloid (Aβ) protein following of the cleaving of the trans membrane amyloid precursor protein (APP), partially by means of a β secretase called $BACE_1$. Approaches to the inhibition of $BACE_1$ require an understanding of the means by which it operates and of means by which it is inhibited. $BACE_1$ is an aspartic protease which attacks and cleaves one bond of a relatively labile tetrahedral intermediate in APP. The description of the means of cleaving has not been without controversy although the cleaving mechanism has now been comprehensively described. Such a description has, likewise, not been without controversy not least because the mechanism of cleaving is energetically unfavourable. This energy barrier is overcome by means of the quantum effect known as hydrogen tunneling. Further, attempts at inhibition have been fraught with difficulties but all follow the same basic approach - to model the inhibitor on the corresponding sequence of the cleaved protein but to insert a less labile intermediate at the scissile bond - in other words a peptidomimetic approach. Initial studies were disappointing not least because of the very low bioavailability of inhibitors brought about by the inhibitory actions of the ATB binding-cassette permeability glycoprotein and the cytochrome P450 3A4 protein. Recent studies adopting this approach have been more promising with better inhibitor design possessing characteristics which significantly improve bioavailability whilst at the same time reducing Aβ in the brain and cerebral fluid. It can be concluded that the peptidomimetic

approach is promising.

2 Introduction

Alzheimers disease - AD, was first diagnosed in about 1906 by a neuropathologist called Aloysius Alzheimer. His principal patient, Auguste Deter died in 1906 and in the last few yeas of her life she had exhibited short term memory loss and abnormal behavioural traits. Brain sections of this patient revealed plaques and tangles. At that time the plaques were identified as amyloid (L. *amylum* - starch) because that is what they were believed to be; this was because of the staining techniques available at the time. The tangles were fiberous in nature and since they were found in the brain they were described as neurofibrillary. Neither of these characteristics were (believed at that time to be) present in healthy brain tissue. Alzheimer presented his findings and the rest is history. Crucially the pathology of AD presents with both neurofibrillary fibers or tangles and plaques.

In the UK alone the current estimate is that about ½ million people are suffering from AD. It is believed to be the most common cause of dementia. If this statistic is correct then this represents a sizeable proportion of the population and is clearly of immence concern.

AD is, depending upon which of three schools of thought one subscribes to, a disease characterised by accumulation in the brain of things which the body cannot get rid of. There are a number of amiloyd diseases which are based upon accumulations and they include vCJD and Huntington's disease. In neurological terms these diseases have common general characteristics such as dementia, though the pathology of AD is by no

means straightforward.

In developing strategies to treat AD two possible ideas spring to mind. The first is to find some way of "clearing up" the accumulations and the second is to stop the thing from happening in the first place. It is this second possible strategy which forms the focus of this project. However, as shall be seen in the chapters to follow, much depends upon one's view of the causes and agents of AD.

Whilst the exact pathology of AD is by no means complete, and indeed is the subject of some competing contentions, it is known or, more properly, believed by some, that certain cleaving enzymes play a part in the pathogenisis of AD. Stop the creation or effect of the cleaving enzyme and you might have a good chance of stopping the disease. This project is based around one of these cleaving enzymes which is known as the first (as in the first one to be discovered - in fact there are two which appear to be interlinked) amyloid precursor protein cleaving enzyme. Because it takes effect on a second site of a particular protein (the amyloid precursor protein - there are three sites, α, β and γ) it is known as the beta first amyloid precursor protein cleaving enzyme or $BACE_1$ (for β site Amyloid precursor protein Cleaving Enzyme 1, or base one). $BACE_1$ is a β secretase and is or has been known as memapsin-2 or Asp-2.

The aim of this project is to investigate through literature review and reasoning $BACE_1$, its structure and means of inhibition from a chemical perspective. Somewhat unfortunately because of constraints of time and cost experimental work is not possible in an Open University context.

It is accepted that the concepts and ideas have a firm grounding in biochemistry. However this is a chemistry project. The notion of conformational change, inhibition and structural stability is a key part of any chemical system and it is these questions which it is intended to investigate.

3 What is the extent of AD in the brain and how does it operate? Why is $BACE_1$ important?

The pathogenisis of AD is by no means fixed. There are three schools, the cholinergic, tau and the beta-APP hypotheses.

3.1 The cholinergic hypothesis.

Cholinergic means working like choline. If a substance or thing is cholinergic then that substance or thing will release acetylcholine when stimulated or will be activated by acetylcholine or acts like acetylcholine. Choline (Gk. *khole* - bile) comprises the *N*, *N*, *N* - tri methyl ethanol ammonium cation. Acetylated choline (choline with the addition of a CH_3COO group) or acetylcholine - AC is a well known neurotransmitter (amongst other transmission capabilities).

Francis *et al* (1999) in a highly critical review article stress the existence of all three schools but at one time in the late mid-1970s the cholinergic hypothesis was in or rose to great favour. The reason for this was because AD patients exhibited deficits in the enzyme responsible for the synthesis of AC, (acetyl choline transferase - ACT). ACT (2fy2.pdb) is a globular protein which catalyses a reaction between acetyl Coenzyme A - acetyl

CoA, and choline to yield AC.

Francis *et al* (1999) (drawing from Bartus *et al* (1982)) stated the hypothesis in the following way:-

> "... [it is the idea that] degeneration of cholinergic neurons in the basal forebrain and the associated loss of cholinergic neurotransmission in the cerebral cortex and other areas ... [which] contribute[s] ... significantly to the deterioration in cognitive function seen in patients with Alzheimer's disease."

What was being said was that cholinergic neurons were being degenerated or depleted and that this negatively affected synapse formation. Synapse formation is essentially where one neuron transmits something to another neuron or cell. The pathway can be electrical or chemical and in the case of cholinergic neurons it is chemical - operating via AC. The communications between nerve cells or synapses were not being formed and this was said to be due to the loss of cholinergic neurons. Hence, so the theory went, the challenge was to inhibit the depletion of cholinergic neurons. This could be achieved by increasing available AC or inhibiting AC inhibitors such as cholineesterase..

The problem was that attempts to regenerate AC or inhibit cholinesterase by target drug treatments proved ineffective. This, of course, does not mean that the cholinergic hypothesis is at all bad but rather that strategies for approaching the problems which the hypothesis illustrates are not effective. Perhaps diplomatically Francis *et al* (1999) stated that

> " ... the hypothesis predicted that ... drugs [having an action similar to that of acetylcholine] would improve cognitive function. This prediction was not fully realised with compounds such as physostigmine and tacrine, probably because the emergence of side effects that may have constrained the dosing regimen to sub-efficacious doses."

This is not the same as saying that the proposed drugs were ineffective, it was just that they were unsafe. It should however be said that as recently as 2003 use of cholinesterase inhibitors were the only available treatment of AD (Lanctôt *et al* (2003)) although their efficacy had yet to be quantified. The reliance upon cholinesterases as pathogenic agents in AD was strictly outside the choligenic hypothesis since the hypothesis was concerned with the degeneration of choligenic neurons and not the esterification of AC once it was engaging in synaptic behavior. The esterification adjunct works on the principal that whilst it might be accepted that AC formation is inhibited by AD the availability of AC can be enhanced by inhibiting its esterification which may be regarded as a form of leaking.

In sum however none of the approaches aimed at dealing with AD on the basis that the cholinergic hypothesis is correct appear to have been completely effective.

3.2 **The tau hypothesis**

Adherents to the tau hypothesis are called tau-ists. Microtubules in neurons are stabilised by a form of glial protein which has a tau function. These glial proteins hold the microtubules together. The microtubule-tau protein complex is the usual formation for microtubules in brain borne nerve cells. However the glial or tau proteins may become phosphorylated (or more commonly hyper phosphorylated) or may be acted upon by effector proteins so that their conformations change and they cease to be glial in their nature. The microtubules become destabilised and, importantly, the tau proteins become polymerised and form tangles (Ávila *et al* (2002)). The

tau hypothesis thus only explains one cause (neurofibilatory tangles comprising helical polymers of abnormally phosphorylated tau protein) - one of two, the other being plaque formation. Tau-ists tend to believe that plaque formation is not a singular feature of AD and, thus, often suggest that neurofibilatory tangles (and their causation) present the best area for research and progress.

Maccioni *et al* (2010) in an opinion article suggest that the tau hypothesis may be summarised in the following way as being:-

> ‘ ... protein components of ... [neurofibillary tangles] and the [polymerised] paired helical filaments ... [of tau proteins which are] hyper-phosphorylated forms of tau ...’

The tau hypothesis has some credence because the accumulation of plaques has ambiguous implications on neuronal functions, thus any theory based upon plaque accumulation is either very complex or is not viable. It is thus that neurofibilatory tangles are said to be the primary pathogenesis.

3.3 **The amyloid hypothesis**

Adherents to the amyloid hypothesis are called baptists (β-APP tists). Baptists believe that the primary pathogenesis of AD is through the generation of plaques.

It is undoubtedly the case that any theory based upon plaque accumulation is either very complex or is not viable. However, there is compelling evidence, even bearing the foregoing in mind, to suggest that plaques have some correlation with improper cleaving of APP. This evidence comes from the fact that the gene for APP is located on chromosome 21.

Individuals with too many of chromosome 21 (trisomy 21 or Down's Syndrome) almost inevitably develop AD by the time they are 40[1] (see Nistor *et al* (2007)).

Importantly two papers published in 2009 went and established a correlation between plaques and the incidence of AD (see Moreno *et al* (2009) and Pigino *et al* (2009)). The baptists seem to have the day ... for now.

3.4 **The amyloid hypothesis - the chemistry**

This project investigates the chemistry of the biopolymers related to the development of AD in the context of the baptist school. For that reason it is necessary to look into the pathogenisis of AD from a baptist perspective.

Moreno *et al* (2009) state the problem as follows:-

> "Clinically, ... AD is manifested as a progressive deterioration of selective populations of neurons affecting particular cognitive domains, with initial symptoms indicating a decline in memory function. From a neuropathology perspective, AD has 2 (*sic*) major characteristics:
>
> > (i) accumulations of extracellular aggregated peptide known as beta amyloid (Aβ), which form the well-characterized senile plaques; and
> >
> > (ii) intracellular accumulation of an abnormally phosphorylated protein, tau, leading to the formation of neurofibrillary tangles."

Hence, ignoring the tau hypothesis, it is the aggregation of plaques (known as beta amyloid or Aβ) which is said to be the neuropathological genesis of the disease - as will be shown this is not necessarily correct. In

[1]Though this is not the personal experience of the author. His first cousin, who has trisomy 21, is 56 years of age - still firing on all cylinders.

investigating the neuropathology it is necessary to ask what does Aβ comprise and how does it get there?

3.5 **What is an amyloid?**

An amyloid was originally defined as any protein which stains blue with the iodine reaction and which under specific circumstances form β pleated sheet fibrils. In more recent times Röcken and Sletten (2003) defined an amyloid as

> " ... an amorphous, eosinophilic[2] proteinaceous deposit that shows a typical green birefringence in polarized light after staining with Congo red, the presence of non-branching linear fibrils of indefinite length with a mean diameter of 10nm, and a distinct X-ray diffraction pattern consistent with Pauling's model of a cross β-fibril."

A cross β-fibril is an oligimer of separate proteins comprising cross β sheets. The word "cross" is imported from X-ray diffraction work where a cross pattern is derived and the expression cross β implies that there is a β sheet. Adding the word "fibril" simply means that a number of amyloidic proteins have polymerised (often believed to be oligomerised) to form fiberous protens. It is believe that this is what the senile plaques are. The polymer precursor is amyloid beta or Aβ.

When oligomerised Aβ forms fibrils and it is believed that this is the means by which plaques are formed (Hoyer *et al* (2008)). Further investigation of the nature of those plaques is outside the scope of this project but it is believed that the plaques themselves are not themselves pathogens, as Glabe and Kayed (2006: S74 & S75) commented:

[2]Meaning is attracted to eosin - a red dye resulting from the action of bromine on fluorescein.

> "It has long been known that the extent of amyloidplaque accumulation does not correlate well with AD pathogenesis ... and that a significant number of nondemented individuals have impressive amounts of amyloid plaques. In transgenic animals and cell culture models, pathologic changes are frequently observed prior to the onset of amyloid deposit accumulation.
>
> "These seemingly conflicting lines of evidence can be reconciled by postulating that amyloid oligomers, rather than the mature fibrils represent the primary toxic species in amyloid-associated degenerative disease."

Importantly Aβ is not itself an amyloid - the oligomers appear to be the toxic agent (Kayed *et al* (2003)). What appears to happen is that Aβ presents itself with an α sheet or α helix structure, which then undergoes a conformational change to include a β sheet structure which in turn oligomerises to form a fibril (Shao *et al* (1999)). It is apparently the β sheet intermediate which is patheogenic.

3.6 **What does Aβ comprise?**

Aβ (1iyt.pdb) was first isolated at the beginning of the 1990s (Talafous (1994)). Aβ is a protein and comprises 39-43 amino acid residues with a C and N end. The amino acid sequence for the 1-40 form is shown below with sequences in red being (in one environment) helices (T indicates a beta turn and S indicates a strand)

```
D A E F R H D S G Y E V H H Q K L V F F A E D V G S N K G A I I G L M V G G V V
  S S S T T H H H H H H H H H H H H H H H H H H H H T T H H H H H H H H H H H H S
```

(Source: PDB database for 1iyt.pdb)

The secondary structure of Aβ is very environment dependent. Ma *et al* (1999) suggest, for instance that not only is the secondary structure solvent dependent but is also dependent upon pH. Further Ma *et al* (1999) were unable to identify any α sheet structure but instead only identified α helix structures. Further Glabe and Kayed (2006) suggest that an α sheet

structure is unlikely. It may be concluded that any case for definite structures of Aβ are either complex or ambiguous.

Aβ is so called because it is a beta protein. Beta proteins are strange to say the least and are characterised by the fact that in an amino acid context, the amino group is bound to the beta carbon of the amino acid. None of the naturally occurring amino acids are beta amino acids.

A typical α- protein is shown as follows:-

A typical β- protein is shown as follows:-

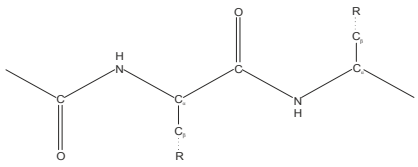

Figure 1 - α Protein

4 What is the genesis of Aβ?

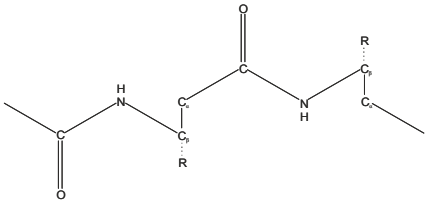

Figure 2 - β Protein

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The following account is taken generally from Karp (2010:64-67). Aβ has a precursor. The precursor is a protein and it is called the Amyloid Precursor Protein or APP. APP comprises three cleaving sites α β and γ and it spans the nerve cell membrane with the NH_2 end in the lumen of the endoplasmic reticulum. How it gets there is beyond the scope of this project. The clevaving sites are subsceptible to cleaving by secretases called α β or γ secretases It is believed that these secretases are also located in part in the cell membrane. Once APP is cleaved at its β and γ sites the result is Aβ, and though the length of the Aβ protein may vary it is usually 40 amino acid residues in length (often called Aβ40). The β secretase is a cleaving enzyme and in conjunction with the γ secretase it cleaves the APP to form Aβ; hence $BACE_1$. The cleaving mechanism is as follows in broad outline:-

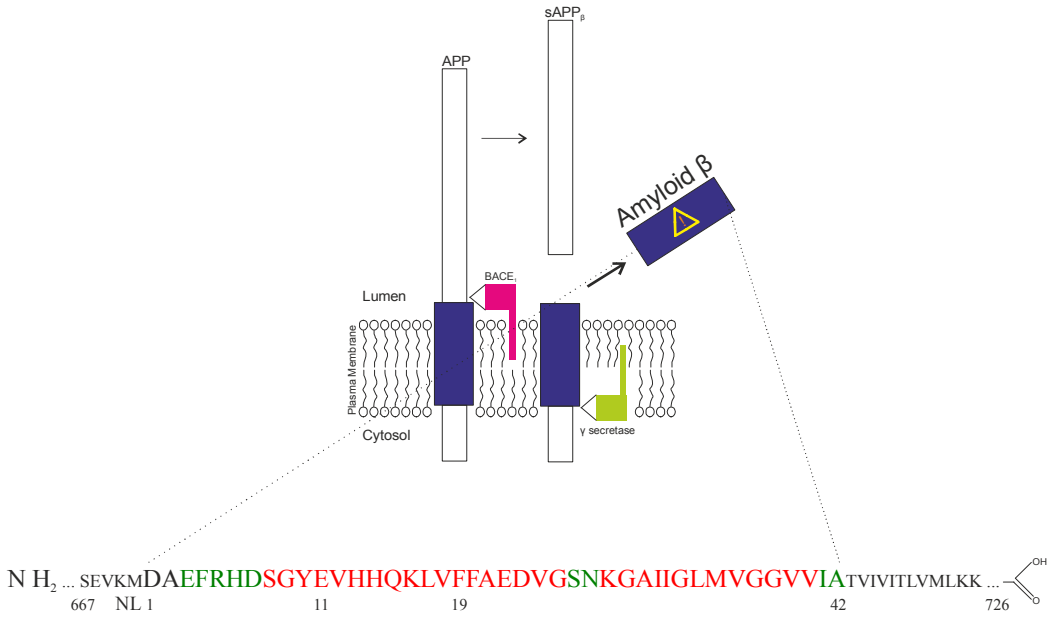

Figure 3 - Aβ cleaving

The lys-met (KM) residues can be substituted by asn-leu (NL) residues in an alternative structure form of APP (called APP_{670NL}). This point (KM/NL) is of extreme importance later on when the OM99 inhibitors are considered.

5 Aspartic Proteases

The L-aspartic acid ($-CH_2-COOH$) and L-glutamic acid ($-CH_2-CH_2-COOH$) residues, aspartyl and glutamyl are the two acids of the amino acid system. The pKa of the aspartyl residue is 3.71. This implies that if the dissociated form exists - as it is known to - then it is predominant over the protonated form only if pH>pKa. Since the normal physiological pH is in the region of 7 the ionised form predominates in normal physiological conditions. Since upon deprototion the charge distribution between the two oxygens becomes delocalised there is a fair amount of ability to form hydrogen bonds and draw charge away from other bonds. This tends to make those, other, bonds more labile and more prone to cleavage. It is this mechanism, often coupled with water which makes the aspartyl residue a particularly good - indeed the best - cleaving candidate. This lead to the development of understanding of aspartic acid proteases; their understanding is important not least because of the part it plays not only in AD but also other diseases such as HIV infection.

Aspartic proteases are highly homologous,

6 $BACE_1$

$BACE_1$ is, as has been said, a secretase. Its identity was initially disclosed by Vassar *et al* (1999: 735 & 736) as follows:-

> "The sequence of the 2526-base pair BACE cDNA revealed an open reading frame of 501 amino acids. The BACE protein has an NH_2- terminal signal peptide of 21 amino acids followed by a proprotein domain spanning amino acids 22 to 45 ... . The lumenal domain of the mature protein extends from residues 46 to 460, and is followed by one predicted transmembrane domain of 17 residues and a short cytosolic COOH-terminal tail of 24 amino acids.
>
> "BACE contains two active site motifs at amino acids 93 to 96 and 289 to 292 in the lumenal domain. Each motif contains the highly conserved signature sequence of aspartic proteases, $D\ ^{T}/_{S}\ G\ ^{T}/_{S}\ ^{3}$ within which the aspartic acid residue is essential for catalytic activity ... . BACE also has four putative N-linked glycosylation sites and six lumenal cysteines, which would allow the formation of up to three intramolecular disulfide bonds ... . Thus, BACE is predicted to be a [homodimeric glycosolated] type I transmembrane protein[4] with the active site on the lumenal side of the membrane, where β-secretase cleaves APP ... ."

As with catalytic proteins $BACE_1$ has active sites or protease domains, that is sites (in this case two - the 93-96 and 289-292 Asp sites) to which molecules become coordinated or bind so that reactions involving those molecules can be catalysed. Usually (though not always) binding takes place over eight amino acid residues of the substrate (which residues are denoted, rather long windedly but with good reason as : S_4-S_3-S_2-S_1*-S_1'-S_2'-S_3'-S_4', where * denotes the cleaving or scissile bond). The corresponding enzyme amino acid residue subsites are denoted by the substitution of P for S. As $BACE_1$ is an aspartic protease it cleaves by means of its aspartic acid residues at its active sites. The cleaving mechanism was believed (Suguna *et al* (1987)) to operate as follows:-

[3]This means the amino acid permutation asp-thr-gly-thr, asp-thr-gly-ser, asp-ser-gly-thr or asp-ser-gly-ser where the aspartic acid and glycine amino acids are constant but the remaining to may alternate between threonine and serine.

[4]Anchored to the lipid membrane and inserted by means of other mechanisms until a stop-transfer sequence on the protein is encountered.

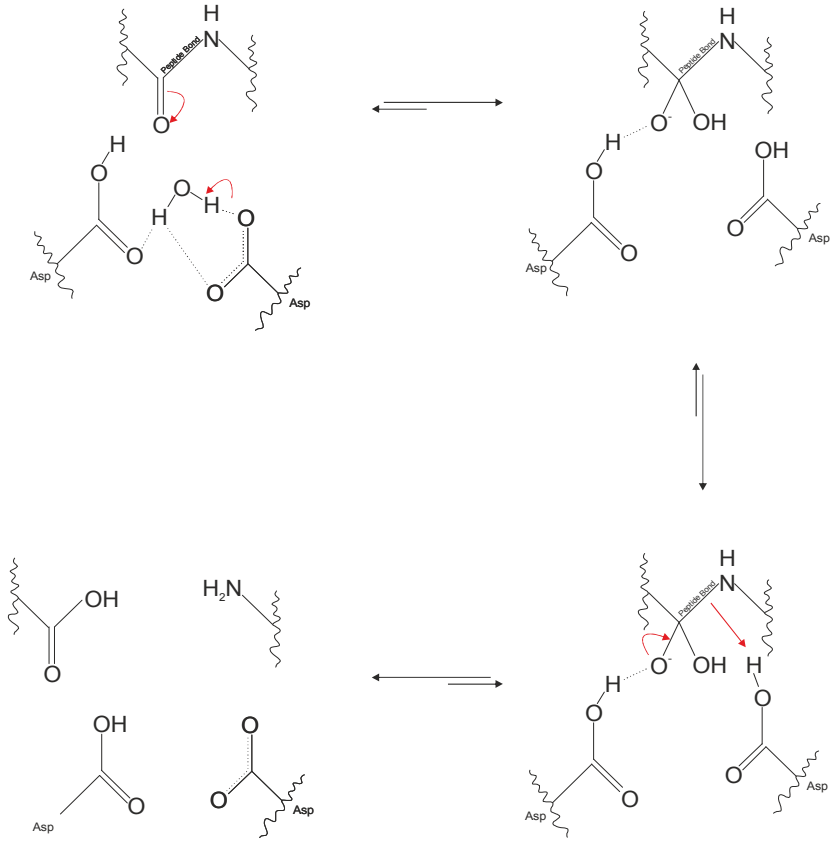

Figure 4 - Suguna Mechanism

Importantly this hydrolitic[5] cleaving mechanism results from highly conserved amino acid residue sequences and that the second part of the process (top right) shows a tetrahedral oxyanion intermediate. The mechanism is an acid-base mechanism in that the coordinated water molecule dissociates to H^+ and OH^- . The right hand aspartic acid residue "activates" the water molecule by the asportation of the cationic proton to leave the hydroxyl anion. Further the tetrahedral intermediate would tend to be more firmly attached to the active site than either the substrate or the product (Patrick (2008: 490)). This may suggest a means of inhibition as

[5]"Hydrolitic" because water H_2O is "broken" or lysated to its acidic and basic forms and the consequences of that breaking is that the acid and bases go on to react elsewhere nearby.

the further processing of the tetrahedral intermediate is rate determining. The amino acids on either side of the scissile bond are usually proline and one of the aromatic residues (phenylalanine, tryptophan or tyrosine). One crucial and important drawback with the Suguna mechanism was that the aspartyl residues were observed to be coordinated with a water molecule but also with a hydrogen atom. This additional coordinating atom is missing from the Suguna mechanism.

Since Suguna *et al* (1987) carried out their research and in the light of the aforementioned criticisms there has been some significant modification of the mechanism (Rose *et al* (1996) and Das *et al* (2006)) known as the Northrop mechanism (Northrop (2001)). Importantly in the Northrop mechanism is the existence of a low-barrier hydrogen bond - the LBHB as the additional coordinating mechanism; this type of bond was established by Piana and Carloni (2000) as a result of calculations, though other researchers had made progress in the area of LBHBs almost a decade previously (see the review by Cleland *et al* (1998)). The essence of the bond is that because of the proximity of coordinating atoms the hydrogen ion is equally bound by both coordinating atoms below and by the water above to yield a cyclic molecule of 8 atoms. Such LBHB bonds are important in that from the situation where hydrogen is preferentially bonded to an adjacent oxygen and only coordinated then the energy from the reorganisation to an LBHB releases energy which can be put to use and may be used to drive a reaction forward. However this hypothesis (known as Cleland's principle after Cleland *et al* (1998) and Cleland (2000)) only works where a LBHB is being formed and not (as here) where it is being lost. The figure below shows the Northrop mechanism in working.

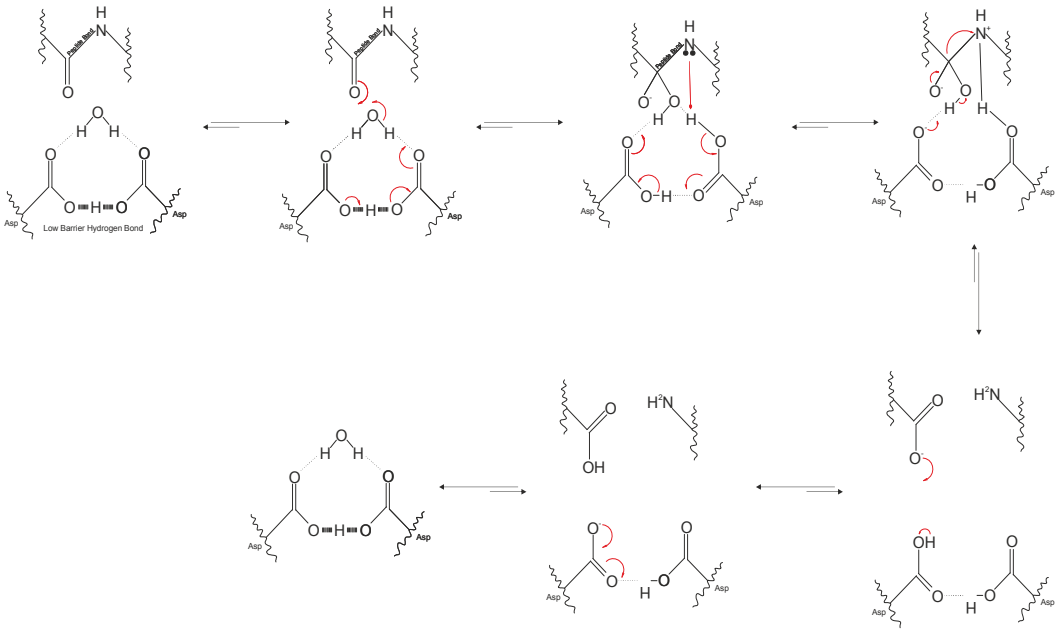

Figure 5 - Northrop mechanism showing LBHB bond (top left) and the effects of hydrogen tunneling (top right).

Importantly the tetrahedral intermediate arises because of hydrogen tunneling and the breaking of the LBHB. The alpha carbon is starved of charge and this, in turn, makes the peptide bond in the tetrahedral intermediate labile. However, as has been said, it is the making of single well hydrogen bonds from the LBHB which is crucial.

The way in which energy barriers are overcome and the movements of hydrogen are attained is by quantum tunneling (Kohen and Klinman (1999)) which relies upon the fact that high energy barriers can be overcome by chance because of the importance of uncertainty in the quantum world. The chance in question is a very low probability that a particle (if particle it be - a matter of some controversy) will be a long way from its current (high probability) position. Hence in the quantum world insurmountable peaks can just be tunneled through (albeit with a low probability of such a thing happening) to get to the other side. This leads one to suppose what might happen if the energy barriers are not so high. It

is likely that this would lead to greater cleaving efficacy and indeed more cleaving. Surprisingly (some may say) the Schrödinger equation[6] predicts this effect without the need for further explanation or modification.

7 $BACE_1$ Inhibition

Having identified how $BACE_1$ cleaves it is necessary to investigate whether that cleaving mechanism can be inhibited. This is best achieved by transition -state analogues; this is because as was suggested by Pauling (1948) and, more latterly, Fersht (1974) the interaction of the enzyme with its substrate at its transition state is the strongest.

Venugopal *et al* (2008) make the following statement as a review of the position in 2008:-

> "Interestingly, BACE-1 is resistant to a broad-spectrum aspartic protease inhibitor, pepstatin A .... In addition, the HIV inhibitors as well as renin inhibitors failed to inhibit the enzyme ... . This led to the conclusion that the active site of the enzyme was different from other aspartyl proteases. From the elucidation of the protein structure and activity assays, it is now possible to know more about the catalytic as well as inhibitory mechanisms. Many different types of assays were invented to tackle this problem; one of the more successful ones was a fluorogenic assay where inhibitors for both BACE-1 and BACE-2 have been described ... . Peptidomimetic statin inhibitors were initially developed based on the sequence of the Swedish mutation of APP[7]. However, other inhibitors were developed based on the substrate specificity of this enzyme, including its peripheral sites to probe its structure as described earlier ... . Some of these inhibitors are very effective in vitro and reduce

[6]This equation is a dynamic equation based upon energies and which is hetrodox when considered against classical mechanical systems based upon point masses and position (such as those predicted, fairly well, to operate in accordance with Newton's Second Law - F=dS/dt, where F is force, S is momentum and t is time). Newton's approach will predict where certain kinds of mass are positioned; however in the quantum world Schrödinger's equation will predict where certain kinds of particles (if particles they be) are likely to be positioned. This adds some fuzz to the question but also results in some very beautiful reasoning.

[7]Swedish APP is a particularly insidious form of APP.

> secretion of Aβ in cultured cells ... . However, drug development has still been hindered by the lack of inhibitors that can be successfully delivered to the brain. Nonetheless, there is a very large effort from pharmaceutical companies and academia to overcome this obstacle. The development of BACE-1 inhibitors is indeed a very large topic, placing it beyond the scope of this review ... ."

Such approaches (towards blocking $BACE_1$ action but, note, not production) are not new - see for instance, Maiorini *et al* (2002) where it is suggested that drugs mimicking transition state intermediates would provide a basis for a way forward. Specifically they reported that a peptidomimetic (mimicking a peptide) inhibitor showed the most promising avenue for further research. However by 2008 at least this approach has all but run out of road. Pepstatin A seemed to be the most promising way forward.

7.1 **Pepstatin A**

Statine (from -stat, stabiliser and -in, neutral) is a gamma amino acid and is not naturally produced. It appears twice in pepstatin and forms the basis for its name - a neutral stabiliser of peptides; it has the sequence Isovaleryl-Val-Val-Sta-Ala-Sta. Importantly, whilst pepstatin is not peptidomimetic it is close to being so.

Marciniszyn *et al* (1976) were the first researchers to establish that pepstatin A had inhibitory effects on acid protases. As research progressed it became apparent that statyl or statyl like residues represented a way

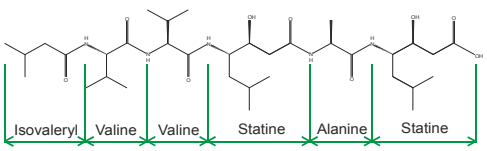

Figure 6 - Pepstatin A

forward (Maorini *et al* (2002)) of the sort H_2N-R-Val-Asn-Sta- ... . However riches and glory did not follow and the efficacy of statine based inhibitors was not sure gone. In a review article Baxter and Reitz (2005) summarised research for statine based compounds; in fact the review concerned all known $BACE_1$ inhibitors - it is just that most were statine based. That article highlighted two important breakthrough papers. The first concerned the actual identification of statines as a potential inhibitor (Sinha *et al* (1999)) and the second a compound known as OM[8]99 (Gosh *et al* (2000) and Hong *et al* (2000), though A.K. Gosh contributed to both papers). Gosh *et al* (2000) - Gosh *et al*, suggested the OM99 inhibitor be based upon the Swedish APP substrate site[9].

7.2 **OM99**

So the logic went - if Swedish APP was such an excellent fit to $BACE_1$ then that would be a good starting point to design irreversable inhibitors. The 2 × 5 substrate identification protocol dictated that the Swedish form of APP would have the following sequence

P_5	P_4	P_3	P_2	P_1	P_1'	P_2'	P_3'	P_4'	P_5'
ser	glu	val	asn	leu	asp	ala	glu	phe	arg

where the double line indicates the cleaving site. Gosh *et al*'s starting point

[8]OM for **O**klahoma **M**edical Research Foundation.

[9]The Swedish APP substrate site had the amino acid residue sequence SEVNL||DAEFR compared to non-Swedish APP substrate site SEVKM||DAEFR where || is the cleaving point. For an interpretation of the amino acid codes see http://en.wikipedia.org/wiki/Amino_acid.

was to propose a simplified 7 residue polypeptide: val-asn-leu||asp-ala-glu-phe where || is the proposed cleaving site or scissile bond. However he then proposed a couple of modifications being:-

(1) the substitution of ala for asp at the P_1' site. This was done for two reasons being that ala is preferred as a P_1' - S_1' binding residue thus increasing the binding energy but also the polarity of the residue is reduced which improves lipophilicity which, in turn, makes the blood/bran barrier[10] more permeable.

(2) the insertion of a hydroxyethylene transition-state isostere between P_1 - P_1' (the normal APP cleaving site).

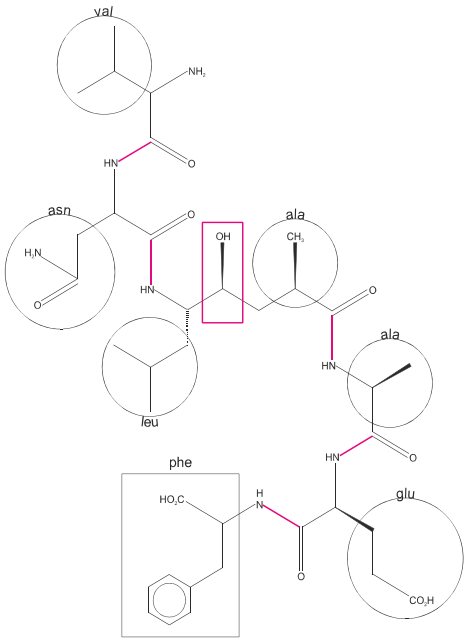

Figure 7 - OM99-1 (red bonds are peptide bonds)

[10]A chemical mechanism where blood vessels in the brain are surrounded by tightly packed, charged, endothelial cells which hinder low lipid soluble, large and charged molecules from passing into the brain. Hence small, lipid soluble and uncharged molecules may pass.

This insertion mimics the transition intermediate required in the Northrop mechanism.

This lead to Gosh *et al*'s proposal of OM99-1, based upon the relevant Swedish APP amino acid sequence with the hydroxyethylene isostere inserted. Hydroxyethylene, ethenol (*cf* ethanol - the cause of many a broken home!) or vinyl alcohol (CH_2CHOH) tautomerises to acetaldehyde and (more importantly) the outward facing hydrogen atom of the alcohol group is positively charged; as such the hydrogen atom has a negative affinity and can form hydrogen bonds (which is characteristic of an alcohol group). The reason it is isosteric with ethenol is because it is part of a chain and the hydroxyl carbon is also an aliphatic carbon.

The effect of the introduction of the hydroxyethylene isostere is to introduce a crucial difference which is that the C-C bond between the alanine residue and the hydroxyethylene isostere is less labile than the corresponding C-N bond. This may be because the bond enthalpy for a C-C bond is $+348 \times 10^3$ J mol^{-1} whereas it is $+293 \times 10^3$ J mol^{-1} for the C-N bond).

Gosh *et al* went on to propose that a further addition of a Glutamic acid residue at the p1 site might yield further potency. However, and what was important, was the fact that ethenol isoteres were of importance.

Here Niu *et al* (2010) take up the story by showing that as recently as 2010 the use of ethenol isoteres was still an area of focus and indeed Dineen *et al* (2012) was encouraging but nevertheless pessimistic, they said:-

> "In previous articles, we have described the synthesis and evaluation of a series of $BACE_1$ inhibitors featuring a hydroxyethylamine (HEA) scaffold as the transition state isostere to bind the critical catalytic aspartic acid residues of the $BACE_1$ enzyme. While select compounds from these series did demonstrate the ability to reduce central Aβ concentrations following peripheral dosing in Sprague.Dawley ... rats, the pharmacological effects of these compounds were limited by their low intrinsic stability ... and poor penetration into the CNS as a result of P-glycoprotein ... mediated efflux. Consequently, significant CNS[11] access and efficacy required either high doses or codosing with a CYP 3A4 inhibitor."

Essentially what Dineen *et al* were saying was that ethenol isoteric based inhibitors were unstable and also stopped from being in or at the areas where they were required because of P-glycoprotein[12] mediated efflux. CY P 3A4 inhibitors are inhibitors to inhibit cytochrome p450 3A4, which coordinated with P-gp.

In their research Dineen *et al* (2012) were able to develop a sensible $BACE_1$ inhibitor which was also able to inhibit CY P 3A4 and which reported significant reduction of Aβ in the brain and cerebo spinal fluid. It may be that an effective treatment is possible after all.

[11]Central Nervous System.

[12]P-glycoprotein or P-gp is part of the cell's rubbish collection and disposal machinery. P-gp - P for permeability - is membrane bound and collects detritus and transports it out of the cell. This means that less or none of the drug is available (called bioavailability) - it arrives, is turned around and marched out again.

8 References

References in the text are in the form Armen *et al* (2004). This citation tallies with the key in this list of references which, in the example given, refers to the paper Armen, R.S., DeMarco, M.L., Alonso, D.O.V. and Daggett, V. "Pauling and Corey's α-pleated sheet structure may define the prefibrillar amyloidogenic intermediate in amyloid disease" Proceedings of the National Academy of Sciences of the United States of America 2004; **101**(32): 11622-11627. Where the authors are a pair then both authors surnames will be cited. Successive annual citations are denoted by the use of lowercase a, b, c a d so on. In the text where a specific page is referred to then the citation will add a page number preceded by a colon to the year of publication, such as Armen *et al* (2004:11624). Emboldened numbers are references to the volume of publication. All journals are peer reviewed unless an astrisk preceds the citation.

Daughter comment articles appear immediately after the father article.

Books are shown with italic titles.

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