Protein lipidation of BACE

R.B. Parsons1 and B.M. Austen
Department of Basic Medical Sciences, St. George’s University of London, Cranmer Terrace, Tooting, London SW17 0RE, U.K.

Abstract
Our research has concentrated upon the protein lipid modification of BACE [β-site amyloid precursor protein cleaving enzyme (β-secretase)], of which very little is currently known. Lipidation influences the production of Aβ (amyloid β-protein) by promoting the dimerization of BACE.

Introduction
A considerable amount of AD (Alzheimer’s disease) research is focused upon the production of Aβ (amyloid β-protein) in AD by BACE [β-site APP (amyloid precursor protein) cleaving enzyme (β-secretase)] [1]. The production of Aβ by the sequential cleavage of APP by BACE and γ-secretase [1] and its aggregation into senile plaques is the basis of the amyloid hypothesis of AD [1]. BACE is localized to acidic intracellular compartments such as the endosome and trans-Golgi network [2]. It is from these domains that BACE is recruited into cholesterol-rich rafts of the plasma membrane where it interacts with APP [3].

Most of the interest in BACE cleavage of APP is centred upon its regulation by cholesterol. Research in our laboratory first demonstrated that LOVA (lovastatin) reduced the production of Aβ in cells transfected with APP Swedish double mutation, which could be overcome by the administration of endogenous cholesterol [4]. Studies by us and other groups have shown that cholesterol influences the glycosylation of BACE without influencing its activity or APP cleavage site selectivity, which in turn influences its recruitment into cholesterol-rich rafts [5]. This is supported by studies, which show that patients on long-term statin treatment are 7-fold less likely to develop AD [6], thus making BACE–lipid interactions attractive therapeutic targets.

BACE palmitoylation does not influence the association of BACE with APP

However, there are a number of modifications of BACE which to date have not been investigated in any depth. There are a number of targeting motifs present within the C-terminal region of BACE [7], in addition to post-translational modifications such as phosphorylation and glycosylation [5]. BACE is palmitoylated on three cysteine residues within the C-terminal region, which regulate its recruitment into cholesterol-rich rafts [7]. Protein farnesylation is usually a precursor to protein palmitoylation and promotes membrane retention [8]. There has been little research into the role of protein lipidation in the production of Aβ. We have shown that LOVA prevents the palmitoylation of BACE, resulting in the inhibition of its association with cholesterol-rich rafts [9]. Aside from this however, little has been reported.

In order to address this, we have investigated the effect protein lipidation has upon Aβ production. Using cerulenin, an inhibitor of fatty acid synthase, to inhibit BACE palmitoylation (Figure 1A), we were able to demonstrate that palmitoylation does not influence the association between BACE and APP. While cerulenin decreased total Aβ production and secretion, it had no effect upon Aβ remaining within the cell (Figure 1B), indicating that BACE is still able to associate with APP. Cerulenin also prevented the association of BACE with cholesterol-rich rafts (Figure 1C), leading to the conclusion that palmitoylation promotes the recruitment of BACE into cholesterol-rich rafts thereby influencing Aβ production.

Farnesylation is involved in the association of BACE with APP

Isoprenylation is an important feature of proteins, with approx. 2% of the proteome undergoing isoprenylation [8]. Isoprenylation consists of farnesylation or geranylgeranyl-ation, and proteins which undergo these modifications contain one of three consensus motifs in their C-terminal regions: CaaX, CXC and CC– [8]. These sequence motifs make the prediction of prenylated proteins relatively simple. As farnesylation is usually a prerequisite for palmitoylation [8], farnesylation may also be important in the BACE cleavage of APP. Recent studies have shown that isoprenoids influence Aβ production [10]. However, BACE does not contain the necessary prenylation sequence motifs, but there is evidence that BACE is associated with other proteins, such as phospholipid scramblase 1, nicastrin and BRI3 (brain protein I3) [11], the farnesylation of which may therefore conceivably influences Aβ production.

We were able to demonstrate that farnesylation is involved in the association of BACE with APP and is not a prerequisite for palmitoylation. Moreover, we showed that BACE is not directly prenylated, but that it associates with a small (53 kDa) protein that co-purifies with nickel affinity-purified BACE (Figure 2A). Inhibiting farnesylation...
It is likely that association with BRI3 stabilizes BACE and stabilization of proteins within neuronal membranes enrichment within the brain suggests a role in the anchorage unknown function, whose membrane-bound localization and candidate. BRI3 is a member of the BRI family of proteins of necessary motif (CaaX) [12] and is therefore the most likely prenylation motifs. This revealed that only BRI3 contains the sequences of those proteins which associate with BACE for recruitment of BACE into cholesterol-rich rafts (Figure 1C).

BACE palmitoylation (Figure 1A). CVFM also inhibited the production within the cell (Figure 2B) without inhibiting and release (Figure 2A) of Aβ. (C) Effect of cerulenin and CVFM upon BACE trafficking. In cells incubated in medium only (left panel), staining was predominantly associated with the plasma membrane with a punctate appearance (b), with no intracellular staining (a). In the presence of cerulenin (5 μg/ml, middle panel) and CVFM (0.1 μM, right panel), there was significant intracellular staining associated with the presence of BACE within the trans-Golgi region (b). Plasma membrane staining was still present, but had lost its punctate nature (a).

using the peptidomimetic inhibitor CVFM (Cys-Val-Phe-Met) resulted in a dose-dependent decrease in Aβ release and production within the cell (Figure 2B) without inhibiting BACE palmitoylation (Figure 1A). CVFM also inhibited the recruitment of BACE into cholesterol-rich rafts (Figure 1C).

In order to identify this 53 kDa protein, we analysed the sequences of those proteins which associate with BACE for prenylation motifs. This revealed that only BRI3 contains the necessary motif (CaaX) [12] and is therefore the most likely candidate. BRI3 is a member of the BRI family of proteins of unknown function, whose membrane-bound localization and enrichment within the brain suggests a role in the anchorage and stabilization of proteins within neuronal membranes [12]. It is likely that association with BRI3 stabilizes BACE within the membrane and promotes its association with APP. What makes BRI3 interesting is that mutations in BRI2 are responsible for British and Danish familial dementias [13]. Although no known mutations occur in BRI3, they do exhibit significant (43%) homology [12]. The 53 kDa protein we observed is somewhat larger than BRI3; however, it may represent either a stage in the assembly of the BACE complex or another as yet unidentified protein. Our identification of BRI3 as the prenylated protein is only putative, but based upon the available evidence, it is the most likely candidate. Ongoing work using antibodies specific to BRI3 will clarify this further.

**Palmitoylation and farnesylation are critical for the dimerization of BACE**

The inhibition of the recruitment of BACE into rafts is significant, as it is within these membranes that BACE dimerizes to its physiologically active 140 kDa form [14]. The first report that BACE may exist as a dimer came from our own laboratory [15], in which we demonstrated the presence of a 140 kDa BACE-reactive protein in addition to a 70 kDa BACE. Subsequent studies have confirmed this
Dimerization within the membrane is mediated by the C-terminal region of BACE [3,14]. Palmitoylation of the three cysteine residues within this C-terminal region may therefore regulate this process as well as BACE’s association with the N-terminal region of BRI3, which also occurs via its C-terminal region [11]. Farnesylation of BRI3 may retain this complex within the membrane, indirectly providing the second lipid anchor commonly found in membrane-bound proteins [8].

Inhibiting palmitoylation and farnesylation does indeed prevent the dimerization of BACE, resulting in a monomeric protein of approx. 70 kDa (Figure 2C). The dimerization of BACE is therefore not a simple process, but is dependent upon lipid modification to promote its recruitment and retention into the membrane compartments where dimerization occurs. What is also clear is that the current interest in the regulation of Aβ production by statins via cholesterol may not provide the full picture, and that attention must also be focused upon those other pathways that statins influence.

**Conclusion**

Our studies have addressed somewhat the imbalance in BACE research. It also opens up new and exciting therapeutic avenues for the treatment of AD. By selectively targeting BACE palmitoylation and associated farnesylation, it may be possible to design drugs that are more efficacious than statins for the treatment of AD.

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**References**


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