Activation of Phospholipase A_2 by Amyloid β-Peptides *in Vitro*†

Jukka Y. A. Lehtonen, Juha M. Holopainen, and Paavo K. J. Kinnunen*

Department of Medical Chemistry, Institute of Biomedicine, University of Helsinki, Finland

Received January 22, 1996; Revised Manuscript Received April 22, 1996⊗

ABSTRACT: Amyloid β-peptides (Aβ) are centrally involved in the pathogenesis of Alzheimer's disease. Using secretory phospholipase A_2 (PLA_2) from porcine pancreas as a model and in the presence of a limiting Ca^{2+} concentration of approximately 50 nM, the synthetic peptide $A\beta_{1-42}$ activates the hydrolysis of the pyrene-labeled acidic phospholipid analog 1-palmitoyl-2-[(pyren-1-yl)]hexanoyl-*sn*-glycero-3-phosphoglycerol (PPHPG) maximally 2.3-fold, whereas an inhibition of PLA_2 action by 50% on the corresponding phosphatidylcholine derivative (PPHPC) was observed. The above effects were evident at 0.24 nM $A\beta_{1-42}$ corresponding to $A\beta_{1-42}$:phospholipid and $A\beta_{1-42}$:PLA_2 molar ratios of 1:10 650 and 1:7.6, respectively. The presence of 10 mol % 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG) in PPHPC reversed the inhibitory effect of $A\beta_{1-42}$ peptide and for these vesicles the hydrolytic activity of PLA_2 toward the fluorescent phosphatidylcholine was enhanced ∼1.8-fold by $A\beta_{1-42}$. In contrast, inclusion of 10 mol % POPG into PPHPG did not influence either the hydrolytic rate toward the latter lipid or the activating effect of $A\beta_{1-42}$. Ca^{2+} concentrations exceeding 15 μM abolished the enhancing effect of $A\beta_{1-42}$ on the hydrolysis of PPHPG whereas a slight activation of PPHPC hydrolysis now became evident. With limiting [Ca^{2+}] preaggregated $A\beta_{1-42}$ enhanced the hydrolysis of both PPHPG as well as PPHPC but the peptide concentrations required were higher by 3−4 orders of magnitude. The synthetic peptide $A\beta_{25-35}$ corresponding to the hydrophobic membrane-spanning segment of the β amyloid precursor protein activated PLA_2 when using PPHPG as a substrate; however, compared to $A\beta_{1-42}$ the extent of activation was less (∼2-fold) and required higher (1 nM) peptide. $A\beta_{25-35}$ did not affect the hydrolysis of the phosphatidylcholine derivative. The hydrophilic peptide $A\beta_{1-28}$ had no effect on PLA_2-catalyzed hydrolysis of either PPHPG or PPHPC under the conditions used in the present study. Interestingly, the above activating effects of $A\beta_{1-42}$ and $A\beta_{25-35}$ on PLA_2-catalyzed hydrolysis of the acidic phospholipid substrate parallel their toxicity on cultured neurons whereas $A\beta_{1-28}$ had no influence either on cultured cells or on PLA_2 activity.

Alzheimer's disease (AD)[1] is a major cause of dementia in aging populations. This disorder is characterized by the progressive accumulation of intracellular neurofibrillary tangles, abnormal extracellular parenchymal senile plaques, and cerebrovascular amyloid deposits (Sisodia & Price, 1995). The clinical severity of the Alzheimer's disease correlates to the extent on amyloid β-peptide accumulation in the cerebral cortex (Cummings & Cotman, 1995). The principal protein component in the latter is constituted by the highly hydrophobic amyloid β-peptide (Aβ) which consists of 39−43 amino acids and is derived proteolytically from the amyloid precursor protein (APP) (Naidu et al., 1995). APP is a large integral membrane protein composed of a short cytoplasmic part, a single transmembrane domain, and a long intraluminal or extracellular domain. Aβ peptide found in the cerebral amyloid deposits corresponds to parts of the membrane-spanning and intraluminal segments, and these peptides differ only at their C-terminus. More specifically, the series of peptides beginning with $A\beta_{1-28}$ vary in the number of their C-terminal residues and thus include increasingly longer portions of the transmembrane domain, Figure 1.

The principal histological finding in AD is a progressive loss of neurons that is most pronounced in hippocampus, cerebral cortex, and amygdala, i.e., regions of brain responsible for memory, cognition, and behavior (Sisodia & Price, 1995). It has become increasingly evident that β amyloid peptides have a causal role in the molecular neuropathology of AD (Iversen et al., 1995). Aβ may contribute to cell death, and several lines of evidence suggest that synthetic peptides corresponding to $A\beta_{1-42}$ and $A\beta_{25-35}$ can trigger the degeneration of cultured neurons through an apoptotic pathway (Loo et al., 1993). Similarly, transgenic animals expressing Aβ exhibit signs of apoptosis in brain regions where the transgene is expressed (LaFerla et al., 1995). Understanding the underlying molecular pathogenesis is crucial in order to find pharmacological means to prevent the progression of this disease. At the moment three potential mechanisms can be proposed, inhibition of the synthesis of Aβ, inhibition or reversal of the aggregation of Aβ, and reduction of the toxicity of already existing Aβ in central nervous system. However, despite intensive research

† This study was supported by the Finnish State Medical Research Council, Biocentrum Helsinki, Sigrid Juselius Foundation, and the Faculty of Medicine of University of Helsinki (P.K.J.K.), and by the Finnish Medical Foundation (J.Y.A.L.). J.Y.A.L. and J.M.H. were supported by the M.D./Ph.D. program of University of Helsinki.

* To whom correspondence should be addressed at Department of Medical Chemistry, Institute of Biomedicine, P.O. Box 8, FIN-00014 University of Helsinki, Finland. Tel: 358-0-191 8237. FAX: 358-0-191 8276. E-mail: paavo.kinnunen@helsinki.fi.

⊗ Abstract published in *Advance ACS Abstracts,* July 1, 1996.

[1] Abbreviations: PPHPC, 1-palmitoyl-2-[10-(pyren-1-yl)]hexanoyl-*sn*-glycero-3-phosphocholine; PPHPG, 1-palmitoyl-2-[10-(pyren-1-yl)]-hexanoyl-*sn*-glycero-3-phosphoglycerol; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol; PLA_2, porcine pancreatic phospholipase A_2; APP, β amyloid precursor protein; Aβ, amyloid β-peptides; AD, Alzheimer's disease.

S0006-2960(96)00148-1 CCC: $12.00

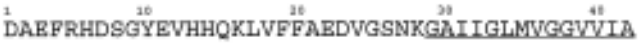

FIGURE 1: Amino acid sequence of the Aβ_{1-42}. The underlined residues correspond to the putative transmembrane domain of APP.

surprisingly little is known about the specific molecular mechanisms of Aβ toxicity. Immediately after solubilization in an aqueous milieu Aβ_{1-42} forms tetramers whereas after prolonged incubation for several days larger aggregates are formed. In contrast, large aggregates are formed by Aβ_{25-35} immediately after its solubilization (Pike et al., 1993). Effects of the "preaggregated" peptides on cultured cells are distictly different from those exerted by the "non-preaggregated" peptides (Pike et al., 1991; 1993). In contrast, "preaggregation" of Aβ_{1-42} abolishes the ability of this peptide to induce pathological responses in cerebrovascular smooth muscle cells *in vitro*. Therefore, distinct mechanisms probably exist for neuronal and smooth muscle cell toxicity (Davis-Salinas et al., 1995). The molecular level mechanism(s) explaining these different effects by preaggregated and non-preaggregated peptides on cellular functions has (have) remained unexplained. Aβ_{25-35} has been suggested to interfere with the cellular redox activity (Shearman et al., 1994). In addition, Aβ increases the content of potentially deleterious H_2O_2 (Behl et al., 1994; Schubert et al., 1995). Both Aβ_{1-42} and Aβ_{25-35} can form Ca^{2+} permeable pores in model membranes, thus raising the possibility that possible changes in cellular Ca^{2+} homeostasis would compromise cell viability (Arispe et al., 1993; Mirzabekov et al., 1994). Changes in tyrosine phosphorylation in neuronal cells occur when exposed to amyloid β-peptides (Zhang et al., 1994).

Interestingly, compared to healthy controls the content of phosphatidylcholine is significantly decreased in autopsy samples from the brains of AD patients. A possible explanation for the latter finding could be an enhanced degradation of phospholipids by phospholipase A_2 (PLA_2) as a consequence of AD (Nitsch et al., 1992). The amphipathic nature of Aβ peptides has been suggested to be crucial for their neurotoxicity (Pike et al., 1993), and both Aβ_{1-40} and Aβ_{25-35} have been shown to bind to negatively charged lipid bilayers (Terzi et al., 1994, 1995). Several membrane-binding peptides influence the activity of PLA_2 (Conricode & Ochs, 1989; Mingarro et al., 1995; Rao, 1992). Phospholipases A_2 are ubiquitously found in the brain tissue, and this group of enzymes plays an important role in neuronal growth (Negre-Aminou & Pfenninger, 1995). In addition, phospholipase A_2 activation has been shown to participate in the regulation of long-term potentiation in hippocampal neurons, i.e., memory and learning (Tocco et al., 1992; Miller et al., 1992). Activation of this group of enzymes has been suggested to occur under hypoxemic conditions (Klein et al., 1993). Furthermore, increased activity of this enzyme has been implicated in schizophrenia (Gattaz et al., 1987). It was therefore of interest to investigate possible effects exerted by amyloid β-peptides on the hydrolytic activity of PLA_2. These lipolytic enzymes have a central role in cellular signal transduction pathways (Dennis et al., 1991; Exton, 1994; Mayer & Marshall, 1993), and they have been suggested also to be involved in the regulation of apoptosis (Hannun & Obeid, 1995; Jayadev et al., 1994). Both intra- as well as extracellular forms of PLA_2 are known, and studies on this enzyme have been aided by its availability in several toxins and pancreatic tissue (Waite, 1987). The primary structures of extracellular PLA_2s from mammalian pancreas, snake venom, and also mammalian intracellular PLA_2s are highly conserved (Waite, 1987; Wery et al., 1991). In keeping with the conserved sequences the three-dimensional structures of these enzymes also show a remarkable similarity, thus suggesting common modes of for instance enzyme−substrate interaction (van den Berg et al., 1993). Accordingly, the readily available pancreatic enzyme is considered to provide a good model for the less easily accessible mammalian intracellular phospholipases A_2 (van den Berg et al., 1993; Cordella-Miele et al., 1990; Waite, 1987). Our results show that the two peptides, Aβ_{1-42} and Aβ_{25-35}, both of which have been shown to exert *in vitro* toxicity, activate the hydrolysis of acidic phospholipids by pancreatic PLA_2 in the presence of limiting Ca^{2+} concentrations whereas the nontoxic Aβ_{1-28} has no effect. This study provides first *in vitro* evidence for the activation of central regulatory lipolytic enzyme by amyloid β-peptides.

EXPERIMENTAL PROCEDURES

Materials. Hepes and porcine pancreatic PLA_2 were from Sigma. Upon electrophoresis in 12% polyacrylamide gel performed in the presence of sodium dodecyl sulfate the enzyme appeared as a single major band stained by Coomassie Blue with a molecular mass of approximately 15 kDa, together with a very faint band of apparent molecular mass of 30 kDa. PPHPG and PPHPC were from K&V Bioware (Espoo, Finland). Aβ_{1-42} (lot no. 508167), Aβ_{1-28} (lot no. 507662), and Aβ_{25-35} (lot no. 510178) were purchaced from Bachem (Bubendorf, Switzerland). The purity of lipids was checked by thin-layer chromatography on silicic acid coated plates (Merck, Darmstadt, Germany) using a chloroform/methanol/water (65:25:4, v/v) solvent system, yielding 0.31 and 0.46 as R_f values for PPHPC and PPHPG, respectively. Concentrations of the fluorescent lipids were determined spectrophotometrically at 342 nm using 42 000 cm^{-1} as the molar exctinction coefficient for pyrene. Aβ peptides were stored in small aliquots at −20 °C until solubilized in distilled water. A separate solution was made for each series of experiments and was used immediately following solubilization. In some experiments, preaggregation of Aβ_{1-42} was first induced by storage of Aβ_{1-42} in capped vials in a 37 °C incubator for 12 days (Pike et al., 1993).

Assay for Phospholipase A_2. Phospholipase A_2 activity was determined by the kinetic assay described previously (Mustonen & Kinnunen, 1991, 1992; Thurén et al., 1985, 1987). Small unilamellar liposomes were formed by rapidly injecting 68 μL of an ethanolic solution of 1.184 mM PPHPG or PPHPC into the buffer (1.6 mL) to yield a lipid concentration of 50 μM (Mustonen & Kinnunen, 1991, 1992, 1993; Mustonen et al., 1993). Of this solution 100 μL was subsequently transferred into 2 mL of 5 mM Hepes buffer, pH 7.4, to obtain a final substrate concentration of 2.5 μM. Aβ peptides were then added, and the contents were equilibrated for 5 min prior to the addition of PLA_2. The reactions with PPHPG were initiated by 50 ng of porcine pancreatic PLA_2. Phosphatidylglycerol was chosen for the acidic headgroup as the binding of different amyloid β-peptides to this lipid has been characterized (Terzi et al., 1994, 1995). Compared to the acidic PPHPG the hydrolytic activity of PLA_2 toward the zwitterionic PPHPC is significantly less (Thurén et al., 1987c), and therefore more enzyme (0.5 μg) was used. Using 50 μM substrate at 25 °C yields

specific activities of 1.66 and 440 μmol min^{-1} mg^{-1} for PPHPC and PPHPG, respectively (Thuren et al., 1987; Mustonen & Kinnunen, 1991). All experiments were repeated three times, with minimal variation (10%−15%).

The progress of phospholipid hydrolysis was followed by measuring pyrene monomer intensity at 400 nm as a function of time using a Kontron SFM 23 spectrofluorimeter with a magnetically stirred cuvette compartment at ambient temperature (~25 °C) while the data were collected through a data aquisition board (DT01-EZ, Data Translation, Marlboro, MA) to a 486 computer. The method used for the quantitation of PLA_2 activity rests on the photophysics of pyrene fluorescence (Kinnunen et al., 1993). In brief, irradiation of this aromatic hydrocarbon at approximately 345 nm generates the monomeric excited state which relaxes back to the ground state by emission with a maximum at approximately 395 nm, the peak energy depending on solvent polarity. If the local concentration of pyrene is high enough (as in the vesicles used as a substrate) the excited monomer may collide with a ground state pyrene forming an excited dimer complex, excimer, which dissociates back to two ground state pyrenes with concomitant fluorescence emission as a broad and structureless band centered at approximately 480 nm. The polarizability of the pyrene moiety can be anticipated to slightly enhance its water solubility so as to make monopyrene derivatives such as PPHPC and PPHPG good choices for PLA_2 assay. Accordingly, these lipids can be used for the assessment of PLA_2 activity as the enzymatic cleavage of the pyrene-labeled acyl chain from the *sn*-2 position yields relatively water soluble product, thus resulting in an enhancement of the monomer emission allowing for a homogenous real time monitoring of PLA_2 (Mustonen & Kinnunen, 1991, 1992; Thurén et al., 1986, 1987c). Importantly, pyrene hexanoate is equivalent in length to a 13 carbon atom hydrocarbon chain, and therefore PPHPC and PPHPG, for instance, are not comparable to short-chain phospholipids. Due to steric reasons pyrene hexanoate may actually be fairly close to palmitate (Lehtonen et al., 1996).

The method used in this study is very sensitive and requires low lipid concentration ($\ll K_m$). Accordingly, the measured specific activities with these substrates are low and must be regarded as apparent only. However, the advantage is that this method allows these types of experiments to be performed at low concentrations of the amyloid β-peptides. The data were analyzed by Origin Software (Microcal, Northampton, MA). The excitation wavelength was 344 nm, and the excitation and emission bandwidths were 10 nm. The assay was calibrated by adding known picomolar aliquots of (pyren-1-yl)hexanoate into the reaction mixture in the absence of the enzyme while detecting pyrene monomer emission. Initial reaction rates were taken from the slopes of the pyrene emission intensity *vs* time data, and enzyme activities are expressed in picomoles of free fatty acid produced per minute in a total volume of 2 mL in the cuvette.

While it would be desirable to study the processes described here using lipids present in neuronal membranes, it should be noted that lipid compositions of these cells are extremely complex and it is difficult to monitor hydrolysis of individual species in such mixtures. Interpretation of the data in terms of molecular level mechanisms would be practically impossible. Therefore, we deem it at this stage a better strategy to start with simple, reasonably well-defined model systems yielding data amenable for reasonable unambiguous interpretation. We would not anticipate major differences between lipids such as POPC and POPG in comparison with PPHPC and PPHPG, respectively. In the probes used the pyrene resides six methylene segments away from the ester bond to be hydrolyzed. Accordingly, neglible perturbation of the enzyme−substrate interaction due to the fluorescent moiety should be evident, and they should thus resonably reliably mirror effects expected on the natural phospholipids present in cellular membranes. The chain length should not influence the catalytic site of the enzyme, acting in the membrane interface where the ester bond resides. The feasibility of the use of pyrenelipids is demonstrated, for instance, by the data of Biltonen and his co-workers who used pyrene-labeled phospholipids to monitor the activity of PLA_2. Although these authors suggested that the enzyme might prefer such lipids over dipalmitoyl phosphatidylcholine, they observed the measured values to correspond to those recorded by pH titration (Burack et al., 1993). Compared to the pH-stat technique, the use of the fluorescent probe allows detection of very low degrees of hydrolysis, thus avoiding any interference due to substantial product accumulation (free fatty acids, lysophospholipids).

A tightly bound Ca^{2+} in the active site is required in the catalytic mechanism of PLA_2. Accordingly, enzymatic activity was completely blocked by ≥ 8 μM EDTA. Unless otherwise indicated the PLA_2 measurements were carried out in the absence of added Ca^{2+}. The residual concentration of this cation is approximately 50 nM due to the presence of Ca^{2+} in the buffering materials and our purified water (Thurén et al., 1990). Under the conditions employed some of PLA_2 molecules present are thus likely to lack Ca^{2+} and be inactive. However, we consider these experimental conditions to mimic reasonably well the intracellular conditions where $[Ca^{2+}]$ is generally in the nanomolar range. Therefore, factors influencing intracellular PLA_2 activity can be simulated.

RESULTS AND DISCUSSION

The amyloid β-peptide, $A\beta_{1-42}$, is ubiquitously found in senile plaques and may be directly involved in the mechanism(s) of neuronal cell death in Alzheimer's disease-afflicted brain tissue. Residues 1−28 are predominantly polar, corresponding to the extracellular domain of APP, whereas segment 29−42 is hydrophobic and constitutes APP's transmembrane segment. Traces in Figure 2 illustrate hydrolysis of PPHPG by PLA_2 in the presence of increasing $[A\beta_{1-42}]$ and in the absence of added Ca^{2+} (i.e., at approximately 50 nM cation). Product liberation begins immediately after the addition of the enzyme without a lag time, and its enhancement is already seen in the presence of 0.1 nM $A\beta_{1-42}$. Figure 3A reveals the activation of PLA_2-catalyzed hydrolysis of PPHPG by $A\beta_{1-42}$ to be sigmoidally dependent on the peptide concentration, with a half-maximal effect at 0.15 nM peptide. Maximally a 2.3-fold increase in the rate of hydrolysis was observed by 0.24 nM $A\beta_{1-42}$ corresponding to a $A\beta_{1-42}$:PPHPG and $A\beta_{1-42}$:PLA_2 molar ratios of approximately 1:10 650 and 1:7.6, respectively. Increasing $[A\beta_{1-42}]$ further (up to 2.5 μM) had no additional effect on the PLA_2 reaction. Increasing concentrations of $A\beta$ lower the surface tension of water up to a critical tension, and at concentrations above (~25 μM) non-covalent aggregates are formed, the latter correlating to the formation

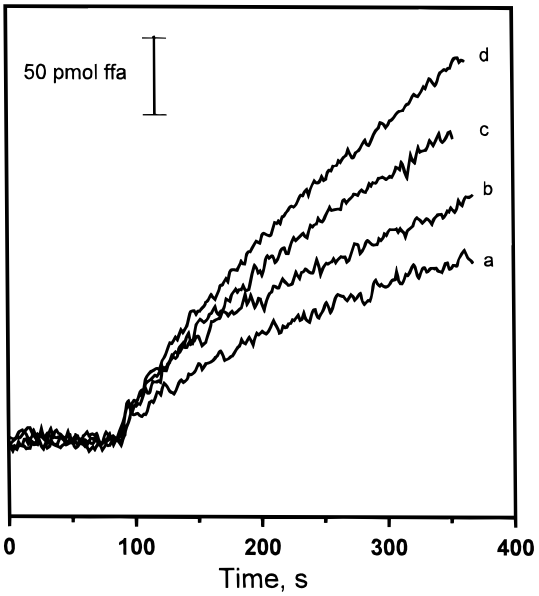

FIGURE 2: Time courses for the release of free (pyren-1-yl)-hexanoate from PPHPG by PLA_2 recorded in the absence (a) and in the presence of 27.5 pM (b), 0.14 nM (c), and 0.28 nM (d) $A\beta_{1-42}$. The medium was 5 mM Hepes, pH 7.4, and the temperature was 25 °C. Substrate concentration was 2.5 μM, and the amount of enzyme was 50 ng in a total volume of 2.0 mL. Only residual $[Ca^{2+}]$ of approximately 50 nM was present.

of a hydrophobic environment that excludes water (Soreghan et al., 1994). However, the concentrations used in the present study are far below the critical concentration of 25 μM. Notably, the increase in PLA_2 activity demonstrated above was evoked at $[A\beta_{1-42}]$ comparable to those concentrations being toxic to cultured cells (Yankner et al., 1990).

Contrasting the enhancement of the hydrolysis of PPHPG an inhibition of PLA_2 activity by maximally 50% was caused by $A\beta_{1-42}$ when the corresponding zwitterionic PPHPC was used as a substrate. The diminished activity was seen in the same $[A\beta_{1-42}]$ range as the activation of hydrolysis of PPHPG, Figure 3B. A lag time of approximately 100 s was evident in the absence of $A\beta_{1-42}$, whereas the presence of the peptide caused the fatty acid release to start immediately after the addition of the enzyme (data not shown).

To study the effect of surface charge 10 mol % POPG was included in PPHPC vesicles. The presence of the acidic phospholipid enhanced the hydrolytic activity of PLA_2 toward PPHPC ~1.8-fold (Figure 4) to 16% of that measured for neat PPHPG. Importantly, in the presence of the acidic phospholipid the hydrolysis of PLA_2 is activated by $A\beta_{1-42}$, in striking contrast to the inhibition observed for the neat zwitterionic lipid. This could be due to changes caused by the $A\beta$ peptide conformation in the presence of acidic phospholipids in the liposomal membranes (Terzi et al., 1994, 1995). In this connection it may be relevant to note that we have recently demonstrated tacrine (1,2,3,4-tetrahydro-9-acridinamine), a drug used in the treatment of the symptoms of Alzheimer's disease, to bind with high affinity to acidic phospholipids (Lehtonen et al., 1996). This drug had little affinity to the zwitterionic phosphatidylcholine. To this end, inclusion of 10 mol % POPG into PPHPG did not affect the hydrolytic rate toward PPHPG, thus suggesting the latter to be slighly preferred by PLA_2 as a substrate, in keeping with observations by Burack et al. (1993). Likewise, POPG did not have any detectable effect on the $A\beta_{1-42}$-induced

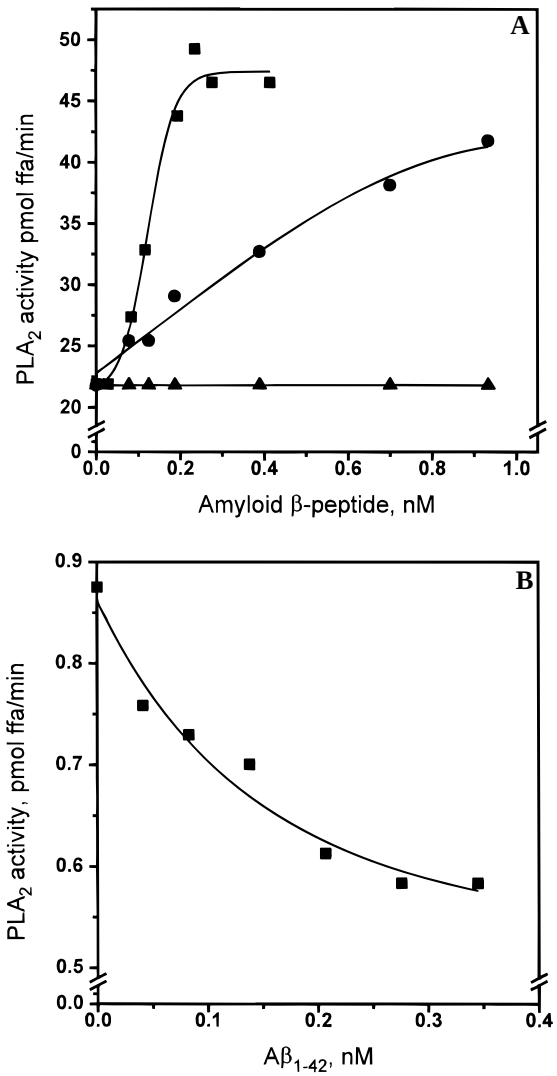

FIGURE 3: (A) Activity of PLA_2 toward PPHPG vesicles in the presence of increasing concentrations of $A\beta_{1-42}$ (■), $A\beta_{25-35}$ (●), and $A\beta_{1-28}$ (▲). Conditions were as described in the legend for Figure 2. (B) Substrate was PPHPC, and the amount of enzyme added was 0.5 μg.

activation of the hydrolysis of PPHPG by PLA_2 (data not shown).

In order to exclude the possibility that trace amounts of Ca^{2+} would be present in the $A\beta$ used, which would then cause the observed enhancement in PLA_2 activity, PLA_2 activity was also measured in the presence of increasing concentrations of EDTA. As shown in Figure 5 both in a medium containing no $A\beta_{1-42}$ as well as in the presence of 0.37 nM $A\beta_{1-42}$, the same EDTA concentration, 8.0 μM, is required to abolish PLA_2 activity, thus indicating the $A\beta_{1-42}$ preparation employed to be virtually free of Ca^{2+}.

In order to determine which part of the peptide is essential for the activation of PLA_2 we then studied the effects of $A\beta_{1-28}$ which corresponds to the extracellular domain of APP and lacks the C-terminal hydrophobic residues. $A\beta_{1-28}$ exerts no toxic effects on cultured neurons (Pike et al., 1993), and it has been shown not to cause plaque formation *in vitro* (Lee et al., 1995). In contrast to $A\beta_{1-42}$, no effect by $A\beta_{1-28}$ on the hydrolysis of either PPHPG or PPHPC by PLA_2 was observed in the concentration range tested (up to 5 μM peptide), Figure 3A. This experiment reveals the membrane-spanning domain of the amyloid β-peptide to be essential

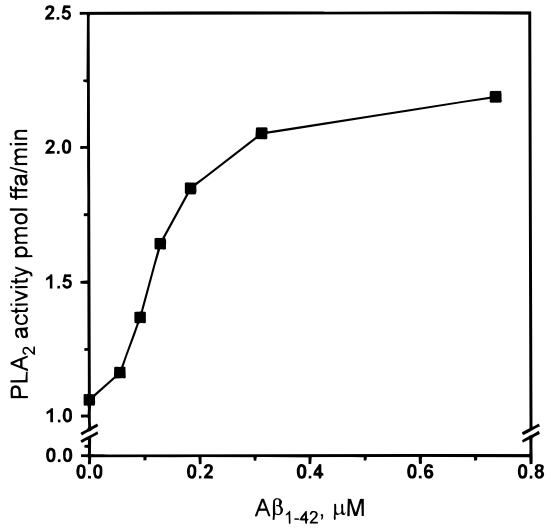

FIGURE 4: Enhanced activity of PLA_2 toward PPHPC in vesicles containing 10 mol % POPG by increasing concentrations of $A\beta_{1-42}$ (■). Amount of enzyme was 50 ng, and the conditions were as described in the legend for Figure 2.

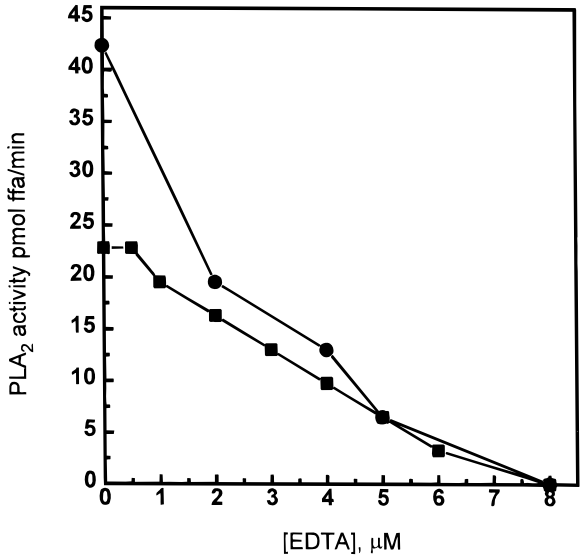

FIGURE 5: Inhibition of PLA_2-catalyzed PPHPG hydrolysis by increasing [EDTA] in the absence (■) and in the presence of 0.37 nM $A\beta_{1-42}$ (●). Experimental conditions were as described in the legend for Figure 2.

for its ability to enhance PLA_2-catalyzed hydrolysis of the acidic phospholipid, PPHPG.

According to cell culture experiments $A\beta_{25-35}$ constitutes the domain of amyloid β-peptide required for both its trophic and toxic effects (Pike et al., 1995; Yankner et al., 1990). The $A\beta_{25-35}$ fragment includes both hydrophilic residues as well as part of the intramembrane domain (i.e., 29−35), and it has been shown to aggregate in water (Pike et al., 1993). Similarly to $A\beta_{1-42}$, $A\beta_{25-35}$ also induces apoptosis in cultured neurons (Loo et al., 1993). As shown in Figure 3A, PLA_2 activity toward PPHPG was elevated maximally 2-fold by $A\beta_{25-35}$. However, compared to $A\beta_{1-42}$ a higher concentration (approximately 0.95 nM) of $A\beta_{25-35}$ was needed for maximal activation. In contrast to $A\beta_{1-42}$, no effect on the hydrolysis of PPHPC by $A\beta_{25-35}$ (up to a concentration of 0.27 μM) could be demonstrated. Interaction with acidic phospholipids facilitates β-sheet formation by $A\beta_{25-35}$, and the binding of this peptide is driven by an electrostatic attraction combined with a weak hydrophobic

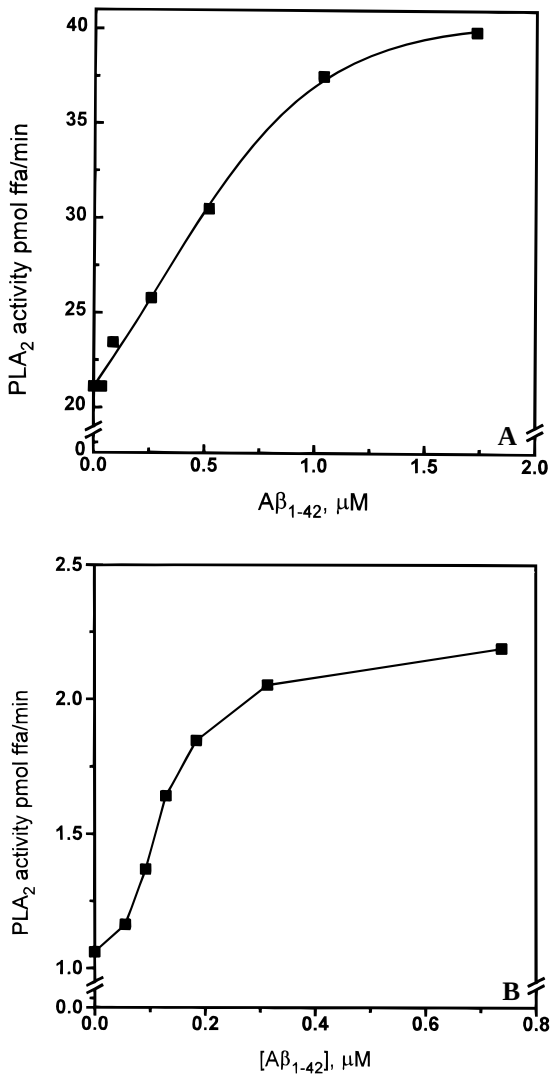

FIGURE 6: (A) Enhancement of the hydrolytic activity of phospholipase A_2 toward PPHPG by preaggregated $A\beta_{1-42}$. (B) Similar experiment as in panel A but using PPHPC as a substrate and 0.5 μg of enzyme. Except for the preincubation of $A\beta_{1-42}$ in an aqueous milieu (see Materials and Methods for further details) prior to its addition to the vesicles in the assay medium, the experimental conditions were as described in the legend for Figure 2.

force (Terzi et al., 1994). Accordingly, the reported lack of interaction of $A\beta_{25-35}$ with the zwitterionic phosphatidylcholine (Terzi et al., 1994) could explain the observed insensitivity of the hydrolysis of PPHPC by PLA_2 to the added peptide.

As was already mentioned above the effects of preaggregated peptides on cells were significantly different from those exerted by peptides added to cells immediately after their solubilization in an aqueous milieu (Pike et al., 1991, 1993; Davis-Salinas et al., 1995). As depicted in Figure 6A preaggregation of $A\beta_{1-42}$ does not abolish the enhancement of hydrolysis of PPHPG by PLA_2 although the peptide concentrations required were approximately 4 orders of magnitude higher. We cannot exclude the possibility that in this case the activation of the enzyme could reflect the presence of small amounts of non-aggregated peptide. Interestingly, preaggregated $A\beta_{1-42}$ enhanced also the hydrolysis of PPHPC by PLA_2, and thus maximally 2.1-fold reaction rates were measured, Figure 6B. Yet, the peptide concentrations needed were significantly higher (3 orders of magnitude) compared to those of the non-preaggregated

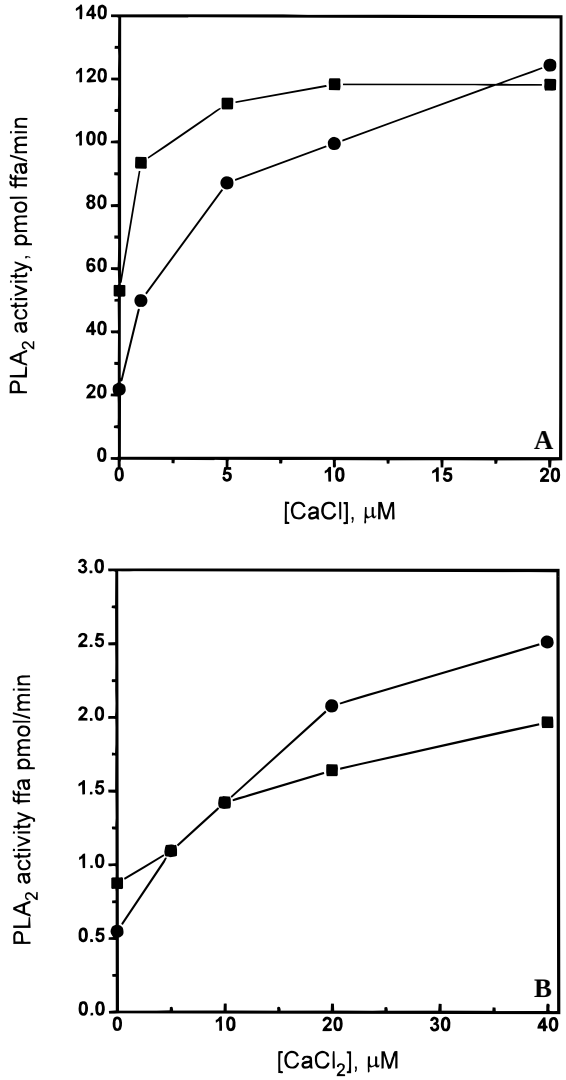

FIGURE 7: (A) Influence of Ca^{2+} on the hydrolytic activity of phospholipase A_2 toward PPHPG in the absence (●) and presence of 0.23 nM Aβ_{1-42} (■). (B) Similar experiment as in panel A but using PPHPC as a substrate and 0.5 μg of enzyme. Otherwise the conditions were as described in the legend for Figure 2.

Aβ_{1-42} causing the inhibition of the hydrolysis of this lipid. Half-maximal activation is observed at peptide:lipid molar ratio of 1:22.7. The effect of preaggregated Aβ_{1-42} on PPHPC hydrolysis is opposite compared to that of the nonaggregated form, and therefore the effect described should be a true property of the aggregated peptide.

Ca^{2+} is essential to the catalytic mechanism of most PLA_2s. Therefore, the above experiments were also repeated at varying [Ca^{2+}]. The concentration of this cation in resting cells is generally approximately 0.1 μM, while in activated cells an increase up to 5 μM may be evident (Alberts et al., 1989). Inclusion of 4.0 μM Ca^{2+} into the reaction medium causes over 4-fold activation of the hydrolysis of PPHPG by PLA_2, Figure 7A. Varying [Ca^{2+}] in the physiological range (up to 5 μM) had no significant influence on the enhancement of PLA_2 activity by 2.35 nM Aβ_{1-42}, and only at [Ca^{2+}] exceeding 15 μM was the influence of this peptide on the lipid hydrolysis of PPHPG completely abolished. Figure 7B shows that 2.35 nM Aβ_{1-42} inhibits the hydrolysis of PPHPC at nanomolar [Ca^{2+}], and increasing the cation concentration further subsequently abolished the inhibitory effect. Accordingly, the data substantiate the notion that the enhancement by Aβ_{1-42} of the activity of PLA_2 with PPHPG substrate cannot be due to contamination of the peptide by Ca^{2+}. Hydrolysis of PPHPC by PLA_2 is enhanced maximally by 1.35-fold at [Ca^{2+}] > 10 μM.

The expression of the catalytic activity of PLA_2s depends on several parameters characterizing the physical properties of the substrate, such as the phase state of the membrane (Op den Kamp et al., 1975), membrane lipid packing defects (Burack et al., 1993; Grainger et al., 1990; Lichtenberg et al., 1986; Sen et al., 1991), and substrate conformation (van den Berg et al., 1993; Thurén et al., 1984, 1987a). The activity of these enzymes also varies as a function of lipid lateral packing in liposomes as demonstrated by the activation of PLA_2 upon osmotic stretching of the substrate bilayers (Lehtonen & Kinnunen, 1995). Finally, the activity of PLA_2 can be triggered by electric fields imposed across the substrate membrane (Thurén et al., 1987b). To this end, the effects of Aβ_{1-42} on the hydrolytic activity of PLA_2 resemble those due to polyamines (Thurén et al., 1986), platelet activating factor (Thurén et al., 1990), adriamycin (Mustonen & Kinnunen, 1991), and phorbol esters (Mustonen & Kinnunen, 1992). In brief, all of these compounds enhance the hydrolysis of acidic phospholipids at low [Ca^{2+}] whereas in the presence of higher [Ca^{2+}] the activation is abolished. Likewise, at low [Ca^{2+}] concentrations the above compounds as well as Aβ_{1-42} inhibit the hydrolysis of phosphatidylcholine. Due to the diversity in the chemical structures of the agents causing the above effects, it is likely that changes in, for instance, surface potential could be involved (Mustonen & Kinnunen, 1991; Mustonen et al., 1993). On the basis of our data, however, we cannot differentiate between an effect of Aβ_{1-42} on the substrate and a direct interaction between Aβ and PLA_2, and a distinction between the two mechanisms must thus await further exploration. We do consider, however, the former to be more likely. Amyloid β-peptides may cause the formation of defects in packing of lipids, which would subsequently promote the binding of enzyme to the membrane surface. Yet, it is also possible that the conformation of the enzyme could be influenced by the presence of the amyloid β-peptides. Judged from the structure of the peptide, it will reside at the interfacial region of the membrane, and therefore effects on the binding of PLA_2 to the membrane might be anticipated. Also, changes in the substrate conformation cannot excluded, but these seem unlikely as the peptide concentration required to induce effects in the system described is very low, 1:10 000 (peptide:lipid ratio). Finally, we cannot offer any conclusive explanation for the differing effects of aggregated peptide and the nonaggregated one.

The importance of membrane binding of the amyloid β-peptides on the pathogenesis of AD has been recently discussed (Yanagisawa et al., 1995). In contrast to Aβ_{1-28}, due to its long hydrophobic segment Aβ_{1-42} can be readily expected to interact also with neutral lipid bilayers. Aβ_{25-35} and Aβ_{1-40} have been shown to bind electrostatically to negatively charged membrane with concomitant changes in their secondary structures (Terzi et al., 1994; 1995). It is not known if these peptides aggregate in bilayers containing acidic phospholipids. Penetration into the bilayer is implicated by the finding that Aβ_{1-40} forms cation channels (Arispe et al., 1993). Together with the observation that Aβ_{25-35} enhances the hydrolysis of the acidic phospholipid but has no effect on the hydrolysis of PPHPC, it is readily

suggested that interaction with membrane is important for the effects of this peptide on lipid hydrolysis by PLA_2. Interestingly, activation of PPHPG hydrolysis and inhibition of hydrolysis of PPHPC occur in the same [$A\beta_{1-42}$] range even though ten times higher enzyme concentrations are used to achieve measurable hydrolysis of the zwitterionic lipid by PLA_2. In addition to possible differences in membrane binding both $A\beta_{1-42}$ and $A\beta_{29-42}$ have been shown to form β-sheet structures while $A\beta_{1-28}$ is essentially random coil (Barrow & Zagorski, 1991; Lee et al., 1995; Hilbich et al., 1991). Peptides influencing PLA_2 activity form aggregates rapidly after their addition to water, and therefore either the aggregated state or a common property driving these peptides to aggregate may also relate to their ability to activate PLA_2.

Direct extrapolation of our present results to the cellular effects of $A\beta$ peptides is certainly ambiguous. Yet, the enhancement of the activity of PLA_2 against acidic phospholipids as well as zwitterionic phospholipids in the presence of acidic phospholipids could provide a novel mechanism by which $A\beta$ may perturb cellular signal transduction pathways, thus exerting major influence on cellular physiology. To this end, enhancement of cellular PLA_2 activity has been suggested to be involved in apoptosis (Shier, 1979; Jayadev et al., 1994; Hannun & Obeid, 1995; Shaposhnikova et al., 1996).

ACKNOWLEDGMENT

The authors thank Birgitta Rantala for technical assistance.

REFERENCES

Alberts B, Bray, D., Lewis, J., Raff, M., Roberts, K., & Watson, J. D. (1989) *Molecular Biology of the Cell*, 2nd ed., Garland Publishing, Inc., NY.

Arispe, N., Pollard, H. B., & Rojas, E. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 10573−10577.

Barrow, C. J., & Zagorski, M. G. (1991) *Science 243*, 180−182.

Behl, C., Davis, J. B., Lesley, R., & Schubert, D. (1994) *Cell 77*, 817−827.

Burack, W. R., Yuan, Q., & Biltonen, R. L. (1993) *Biochemistry 32*, 583−589.

Conricode, K. M., & Ochs, R. S. (1989) *Biochim. Biophys. Acta 1003*, 36−43.

Cordella-Miele, E., Miele, L., & Mukherjee, A. B. (1990) *J. Biol. Chem. 265*, 17180−17188.

Davis-Salinas, J., & Van Nostrand, W. E. (1995) *J. Biol. Chem. 270*, 20887−20890.

Dennis, E. A., Rhee, S. G., Billah, M. M., & Hannun, Y. A. (1991) *FASEB J. 5*, 2068−2077.

Exton, J. H. (1994) *Biochim. Biophys. Acta* 1212, 26−42.

Gattaz, W. F., Köllisch, M., Thuren, T., Virtanen, J. A., & Kinnunen, P. K. J. (1987) *Biol. Psychiatry* 22, 421−426..

Grainger, D. W., Reichert, A., Ringsdorf, H., & Salesse, C. (1990) *Biochim. Biophys. Acta 1023*, 365−379.

Hannun, Y. A., & Obeid, L. M. (1995) *Trends Biochem. Sci. 20*, 73−77.

Hilbich, C., Kisters-Woike, B., Reed, J., Masters, C. L., & Beyreuther, K. (1991) *J. Mol. Biol. 218*, 149−163.

Iversen, L. L., Mortishire-Smith, R. J., Pollack, S. J., Shearman, M. S. (1995) *Biochem. J. 311*, 1−16.

Jayadev, S., Linardic, C. M., & Hannun, Y. A. (1994) *J. Biol. Chem. 269*, 5757−5763.

Kinnunen, P. K. J., Koiv, A., & Mustonen, P. (1993) in Fluorescence Spectroscopy (Wolfbeis, O. S., Ed.) pp 159−169, Springer-Verlag, Berlin.

Klein J., Holler T., Cappel E., Koppen A., & Loffelholz K. (1993*) Brain Res.* 630, 337−340.

LaFerla, F. M., Tinkle, B. T., Bieberich, C. J., Haudenschild, C. C., & Jay, G. (1995) *Nat. Genet. 9*, 21−30.

Lee, J. P., Stimson, E. R., Ghilardi, J. R., Mantyh, P. W., Lu, Y.-A., Felix, A. M., Llanos, W., Behbin, A., Cummings, M., Van Criekinge, M., Timms, W., & Maggio, J. E. (1995) *Biochemistry 34*, 5191−5200.

Lehtonen, J. Y. A., & Kinnunen, P. K. J. (1995) *Biophys. J. 68*, 1888−1894.

Lehtonen, J. Y. A., Holopainen, J. M., & Kinnunen, P. K. J. (1996) *Biophys. J. 70*, 1753−1760.

Lehtonen, J. Y. A., Rytömaa, M., & Kinnunen, P. K. J. (1996) *Biophys. J. 70*, 2185−2194.

Lichtenberg, D., Romero, G., Menashe, M., & Biltonen, R. L. (1986) *Biochemistry 26*, 5334−5340.

Loo, D. T., Copani, A., Pike, C. J., Whittemore, E. R., Walencewicz, A. J., & Cotman, C. W. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 7951−7955.

Mayer, R. J., & Marshall, L. A. (1993) *FASEB J. 7*, 339−348.

Miller B., Sarantis M., Traynelis S. F., & Attwell D. (1992) *Nature 355*, 722−725.

Mingarro, I., Pérez-Payá, E., Pinilla, C., Appel, J. R., Houghten, R. A., & Blondelle, S. E. (1995) *FEBS Lett. 372*, 131−134.

Mirzabekov, T., Lin, M.-C., Yuan, W-L., Marshall, P. J., Carman, M., Tomaselli, K., Lieberburg, I., & Kagan, B. L. (1994) *Biochem. Biophys. Res. Commun. 202*, 1142−1148.

Mustonen, P., & Kinnunen, P. K. J. (1991) *J. Biol. Chem. 266*, 6302−6307.

Mustonen, P., & Kinnunen, P. K. J. (1992) *Biochem. Biophys. Res. Commun. 185*, 185−190.

Mustonen, P., & Kinnunen, P. K. J. (1993) *J. Biol. Chem. 268*, 1074−1080.

Mustonen, P., Lehtonen, J. Y. A., Koiv, A., & Kinnunen, P. K. J. (1993) *Biochemistry 32*, 5373−5380.

Naidu, A., Quon, D., & Cordell, B. (1995) *J. Biol. Chem. 270*, 1369−1374.

Negre-Aminou, P., & Pfenninger, K. H. (1995) *J. Neurochem.* 60, 1126−1136.

Nitsch, R. M., Blusztajn, J. K., Pittas, A. G., Slack, B. E., Growdon, J. H., & Wurtman, R. J. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 1671−1675.

Op den Kamp, J. A. F., Kauerz, M. Th., & van Deenen, L. L. M. (1975) *Biochim. Biophys. Acta 406*, 169−177.

Pike, C. J., Walencewicz, A. J., Glabe, C. G., & Cotman, C. W. (1993) *Eur. J. Pharmacol. 207*, 367−368.

Pike, C. J., Burdick, D., Walencewicz, A. J., Glabe, C. G., & Cotman, C. W. (1993) *J. Neurosci. 13*, 1676−1687.

Pike, C. J., Walencewics-Wasserman, A. J., Kosmoski, J., Cribbs, D. H., Glabe, C. G., & Cotman, C. W. (1995) *J. Neurochem. 64*, 253−265.

Rao, N. M. (1992) *Biochem. Biophys. Res. Commun. 182*, 682−688.

Schubert, D., Behl, C., Lesley, R., Brack, A., Dargusch, R., Sagara, Y., & Kimura, H. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 1989−1993.

Sen, A., Isac, T. V., & Hui, S. W. (1991) *Biochemistry 30*, 4516−4521.

Shaposhnikova, V. V., Dobrovinskaya, O. R., Eidus, L. Kh., & Korystov, Y. N. (1996) *FEBS Lett. 348*, 317−319.

Shearman, M. S., Ragan, C. I., & Iversen, L. L. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 1470−1474.

Shier,W. T. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 195−199.

Sisodia, S. S., & Price, D. L. (1995) *FASEB J. 9*, 366−370.

Soreghan, B., Kosmoski, J., & Glabe, C. (1994) *J. Biol. Chem. 269*, 28551−28554.

Terzi, E., Hölzemann, G., Seelig, J. (1994) *Biochemistry 33*, 7434−7441.

Terzi, E., Hölzemann, G., Seelig, J. (1995) *J. Mol. Biol. 252*, 633−642.

Thurén, T., Vainio, P., Virtanen, J. A., Somerharju, P., Blomquist, K., & Kinnunen, P. K. J. (1984) *Biochemistry 23*, 5129−5134.

Thurén, T., Virtanen, J. A., Lalla, M., & Kinnunen, P. K. J. (1985) *Clin. Chem. 31*, 714−717.

Thurén, T., Virtanen, J. A, & Kinnunen, P. K. J. (1986) *J. Membr. Biol. 92*, 1−7.

Thurén, T., Tulkki, A.-P., Virtanen, J. A., & Kinnunen, P. K. J. (1987a) *Biochemistry 26*, 4097−4910.

Thurén, T., Virtanen, J. A, & Kinnunen, P. K. J. (1987b) *Biochemistry 26*, 5816−5819.

Thurén, T., Virtanen, J. A., Verger, R., & Kinnunen, P. K. J. (1987c) *Biochim. Biophys. Acta 917*, 411−417.

Thurén, T., Virtanen, J. A, & Kinnunen, P. K. J. (1990) *Chem. Phys. Lipids 53*, 129−139.

Tocco, G., Massicotte, G., Standley, S., Thompson, R. F., & Baudry, M. (1992) *Neuroreport* 3, 515−518.

van den Berg, J. J. M., Op den Kamp, J. A. F., Lubin, B. H., & Kuypers, F. A. (1993) *Biochemistry 32*, 4962−4967.

van den Berg, B., Tessari, M., de Haas, G. H., Verheij, H. M., Boelens, R., & Kaptein, R. (1995) *EMBO J. 14*, 4123−4131.

Waite, M. (1987) *The Phospholipases Handbook of Lipid Research*, Vol. 5, pp 155−190, Plenum Press, New York.

Wery, J.-P., Schevitz, R. W., Clawson, D. K., Bobbitt, J. L., Dow, E. R., Gamboa, G., Goodson, T., Hermann, R. B., Kramer, R. M., McClure, D. B., Mihelich, E. D., Putnam, J. E., Sharp, J. D., Stark, J. D., Teater, C., Warrick, M. W., & Jones, N. D. (1991) *Nature 352*, 79−82.

Yanagisawa, K., Odaka, A., & Ihara, Y. (1995) *Nat. Med.* (NY) *1*, 1062−1066.

Yankner, B. A., Duffy, L. K., & Kirschner, D. A. (1990) *Science 250*, 279−282.

Zhang, C., Lambert, M. P., Bunch, C., Barber, K., Wade, W. S., Krafft, G. A., & Klein, W. L. (1994) *J. Biol. Chem. 269*, 25247−25250.

BI960148O