Possible Involvement of Alzheimer Amyloid Precursor Protein and Its Associated Protein Kinase Activity in Signal Transduction Pathway

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Amyloid precursor protein (APP) is one of the major causative agents of Alzheimer's disease and possesses a receptor-like structure with extracellular, single transmembrane, and cytoplasmic domains. To explore the physiological significance of APP in the cell, we focuses on the possible involvement of APP in signal transduction pathways. Using affinity precipitation followed by an *in vitro* kinase assay, we found an APP-associated protein kinase activity in the cytosol/membrane fraction of human cell lines derived from neuroblastoma, glioblastoma, embryo, epidermis, and cervix cancer. The kinase was capable of phosphorylating APP- and/or kinase-associated cellular proteins and of binding to a part of the extracellular domain of APP. The kinase activity was specific for serine residues. These results suggest that APP may function in a form of heteromer with a membrane-spanning receptor-like kinase via binding to the extracellular domain characteristic of other receptor systems.

Keywords: Alzheimer's disease /β/A4 Amyloid precursor protein / Protein interaction / Protein kinase / Phosphorylation / Signal Transduction

Alzheimer's disease is the most prevalent neurodegenerative disease, characterized clinically by progression of memory loss and pathologically by the presence of senile plaques, neurofibrillary tangles, and extensive neuronal loss (1). The major constituent protein (β/A4) of amyloid plaques, which are a hallmark of this disease, is proteolytically derived from the β/A4 amyloid precursor protein (APP). Encoded by a single gene on chromosome 21, APP belongs to a family of alternatively spliced Type I integral transmembrane glycoproteins (Figure 1), the cellular function of which is unknown. After the identification of APP695 (2), which consists of 695 residues, at least 10 isoforms of APP have been identified, resulting from alternative splicing of a single gene. Neurons express high amounts of APP695, while longer APPs (751 and 770), containing a Kunitz-type protease inhibitor (KPI) insert, and those lacking exon 15 (L-APPs) are more abundant in peripheral tissues (1). Genetic studies have revealed that point mutations cosegregate with the disease phenotypes (1). Therefore, structural alterations of APP are thought to be one etiology of the disease, although their molecular mechanisms remain unclear.

As shown in Figure 1, APP structurally resembles a type of cell-surface receptors consisting of a glycosylated extracellular domain with a cysteine-rich region, a single transmembrane

BIOORGANIC CHEMISTRY —Molecular Clinical Chemistry—

Scope of Research

This laboratory was founded in 1994 with the aim of linking biomedical research and clinical medicine. Thus, the scope of our research encompasses the structure/function/regulation of various biomolecules, the pathophysiological significance of divergent bioreactions, the specific abnormalities that cause diseases, and the application of molecular techniques to clinical diagnosis and therapy. Our current interest is focussed on poly(ADP-ribosyl)ation of cellular proteins in relation to carcinogenesis, phosphorylation and NuLS-dependent nuclear localization of proteins related to apoptosis and leukemogenesis, the pathophysiological role of Alzheimer β/A4 amyloid precursor protein (APP) and its associated kinase in signal transduction pathways, the aberrant splicing of the APP gene transcript, and the etiological linkage of Alzheimer's disease to the apolipoprotein ε4 allele.

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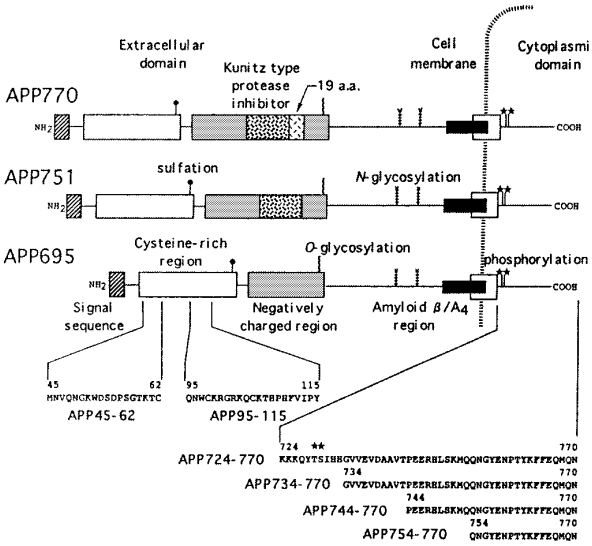

Figure 1. Molecular structure of Alzheimer β/A4 amyloid precursor proteins (APPs). Three major isoforms of APP, APP770, APP751 and APP695, have been identified as a result of alternative splicing of a single gene. The oligopeptides indicated were chemically synthesized using *t*-butoxycarbonyl amino acids and *p*-methylbenzhydrylamine resins, coupled to EAH-Sepharose 4B support by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimido hydrochloride, and used for affinity precipitation followed by *in vitro* kinase assay (5,6).

domain, and a cytoplasmic domain (2). Actually, APP is localized in the cell membrane as an *N*- and *O*-glycosylated form (3). Immunohistochemical studies revealed a patchy and punctate appearance of APP on neurons in rat brain. It has been pointed out that the cytoplasmic domain of APP contains the tetrapeptide sequence, NPTY, which conforms to the consensus sequence, NPXY, required for the rapid endocytosis of the LDL receptor. Subsequently, it has been shown that APP expressed on the cell surface is internalized and delivered to the prelysosomal/lylsosomal branch of the endocytic pathway (3). Ser^{730} and Thr^{729} in the cytoplasmic domain are phosphorylatable

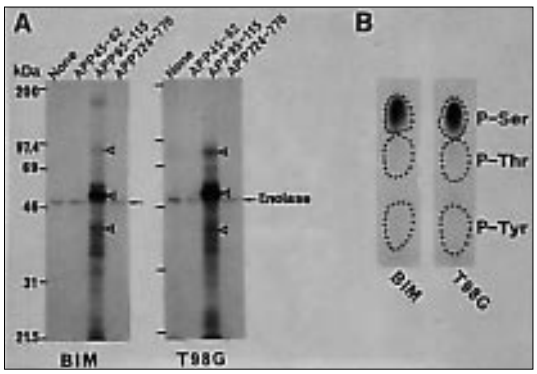

Figure 2. Detection of APP-associated protein kinase activity in human cell lines derived from neuroblastoma (BIM) and glioblastoma (T98G). A, APP peptide-Sepharose beads were incubated with cytosol/membrane fractions from BIM and T98G (6). Recovered affinity complexes on the beads were resuspended in the kinase reaction mixture containing [γ-^{32}P]ATP and exogeneous substrate, enolase, for 10 min at 25°C. After gel electrophoresis, the APP-associated kinase activities were detected by autoradiography. B, phosphoamino acids of proteins phosphorylated by APP-associated kinase were separated by high-voltage electrophoresis and identified by autoradiography (7).

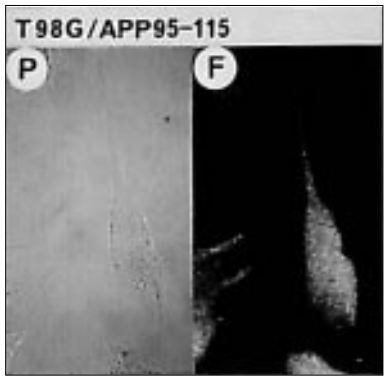

Figure 3. Subcellular localization of APP-associated kinase(s). T98G cells were incubated with fluorescein isothiocyanate-labeled APP95-115 peptide (5). Subcellular localization of the bound peptide (APP-associated kinase) was visualized by fluorescence microscopy (F). The cells were also viewed by phase-contrast microscopy (P).

by protein kinase C and Ca^{2+}/calmodulin-dependent kinase II (1). This phosphorylation might be involved in the regulation of unknown APP function(s) and/or the transduction of unknown signaling(s).

In order to explore the pathophysiological significance of APP, we focuses on the possible involvement of APP in signal transduction pathway. Based on computer analysis of the predicted secondary structures, surface probability, antigen index, flexibility, and hydrophobicity, we synthesized a series of APP peptides for affinity precipitation (Figure 1). By using the affinity precipitation method followed by *in vitro* kinase assay, we discovered an APP-associated kinase activity in the cytosol/membrane fraction from a variety of human cell lines. Figure 2 shows typical data with neuroblastoma cells, BIM, and glioblastoma cells, T98G (4). The APP95-115 sequence in the extracellular domain of APP is a binding site for the kinase or kinase-associated proteins. The APP-associated kinase is capable of phosphorylating APP- and/or kinase-associated protein(s) at serine residues but not tyrosine residues. The kinase requires Mn^{2+} and Mg^{2+} for activation. APP-associated kinase is distributed on the cell membrane (Figure 3). Taken together, we suggest that APP may function in a form of heteromer with a membrane-spanning receptor-like protein with a kinase activity via binding to the extracellular domain as known for other receptor signaling systems.

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