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# NF-κB precursor, p105, and NF-κB inhibitor, IκBγ, are both elevated in Alzheimer disease brain

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**Abstract**

The nuclear factor-κB (NF-κB) signal transduction pathway regulates several vital cellular processes. During our studies of the glycoproteins involved in Alzheimer disease (AD), we found a significant increase of a 45-kDa protein band that was stained by lectin Maackia amurensis agglutinin (MAA). Mass spectrometry and Western blot analyses indicated that this 45-kDa MMA-positive protein was an inhibitor of NF-κB, IκBγ. By Western blot analysis, the levels of both IκBγ and NF-κB precursor, p105, were found to be elevated in AD brain as compared to age-matched controls. Our findings suggest that the NF-κB pathway might be involved in the molecular mechanism of AD.


*Keywords:* NF-κB precursor; IκBγ; Alzheimer disease; Human brain

Alzheimer disease (AD) is a progressive neurodegenerative disorder with multiple etiologies, the molecular mechanism of which is not yet known. Many factors and pathways have been shown to be important to or to be involved in the pathogenesis of AD. Among them, p50/p65 of nuclear factor κB (NF-κB), which regulates transcription of genes involved in several vital cellular processes, has been reported to be activated in AD brain [6,14,15]. However, whether other components of the NF-κB pathway are also deregulated in AD is not known. In this report, we demonstrate that a precursor of NF-κB, p105, and an inhibitor protein of NF-κB, IκBγ, are elevated in AD brain as compared to age-matched controls.

Because protein glycosylation appears to be altered in AD brain [7,8,12], we examined by lectin blots [8] whether the level of any glycoprotein is altered in AD brain. Homogenates of cerebral cortex of six AD (age 73.2 ± 10.1 years (mean ± S.D.); postmortem delay 4.7 ± 3.2 h) and six controls (age 73.6 ± 8.2 years; postmortem delay 5.0 ± 1.2 h) were resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and then either stained with Coomassie blue or with lectin Maackia amurensis agglutinin (MAA) (EY Laboratories, Inc., San Mateo, OA), which is commonly used to stain glycoproteins containing terminal sialic acid–(α2,3)-galactose residues at their glycan component [11]. We found a significant increase in a 45-kDa MAA-positive protein band in AD compared to in controls (Fig. 1A–C). To determine the subcellular distribution of this protein, we prepared various subcellular fractions of cerebral cortex by the classical centrifugation method [13] and stained each fraction with MAA after SDS-PAGE. We found that the 45-kDa band was present predominantly in the nuclear fraction (Fig. 1D).

To identify this 45-kDa protein, we extracted the 100,000 × *g* pellet of homogenates from human cerebral cortex with buffer containing 50 mM Tris, pH 7.6, 0.1% Triton X-100, 0.1% Tween-20, 300 mM NaCl, and a cocktail of protease inhibitors. After probe-sonication and centrifugation, the extracted proteins were separated by high-performance liquid chromatography (HPLC) on an anion exchange Resource Q column (Amersham Biosciences Co.,

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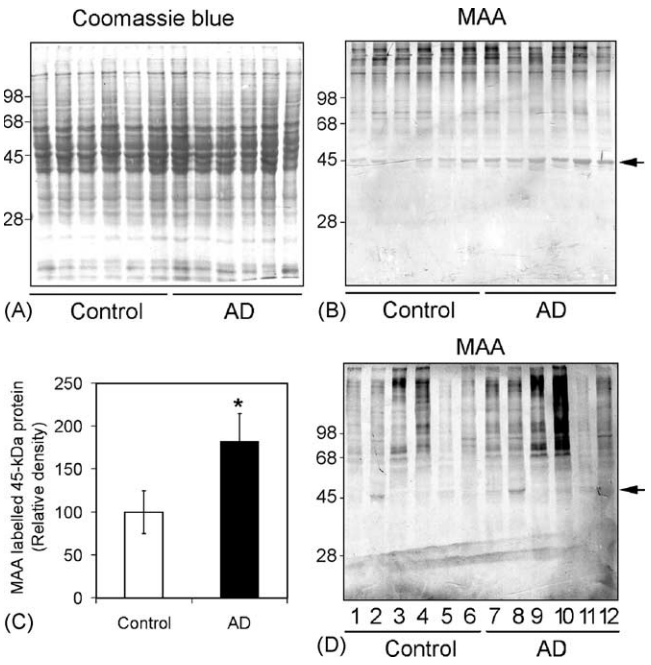


Fig. 1. Coomassie blue and MAA staining of human brain homogenates and various subcellular fractions. (A) Homogenates (5 μg/lane) of cerebral cortex of six AD and six control brains were resolved by 10% SDS-PAGE and stained with Coomassie blue. (B) The proteins (10 μg/lane) from the gel were blotted onto PVDF membrane and stained with lectin MAA (10 μg/ml) that was developed by peroxidase staining using 3,3′-deaminobenzidine. (C) The 45-kDa MAA-positive protein band, marked by an arrow in panel B, was quantitated by densitometry (six AD and six control cases) and is presented as mean ± S.D., $^{*}p < 0.05$ vs. controls. (D) The cerebral cortical proteins were fractioned into various subcellular fractions and then analyzed (5 μg/lane) by lectin blot developed with MAA. Lanes 1 and 7, homogenate; lanes 2 and 8, nuclei; lanes 3 and 9, cytosolic proteins; lanes 4 and 10, microsomes; lanes 5 and 11, synaptosomes; lanes 6 and 12, lysosomes. The arrow indicates the 45-kDa protein that was enriched in nuclear fraction (lanes 2 and 8).

Piscataway, NJ). The eluted fractions were examined by both silver staining and MAA staining after SDS-PAGE. The fractions containing 45-kDa MAA-positive protein were pooled and further separated by 10% SDS-PAGE. The 45-kDa protein was then dissected from the gel and subjected to N-terminal amino acid sequencing that was carried out at the Molecular Resource Facility of the New Jersey Medical School. A sequence of NAGMKHGTMD was identified. Our database search by NCBI BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) indicated that this peptide belongs to the N-terminal part of IκBγ and the central part of NF-κB precursor p105, the C-terminal half of which is homologous to IκBγ [5]. There is no other proteins in the database which is highly homologous to this peptide.

To confirm that the 45-kDa protein, the MAA staining of which was found to be increased in AD brain, was indeed IκBγ, we employed Western blot developed with anti-IκBγ (Santa Cruz Biotechnology, Santa Cruz, CA). This anti-IκBγ recognizes both IκBγ and NF-κB precursor p105. When homogenate samples of human cerebral cortex were used, we observed a weak band at 45 kDa (data not shown) and a clear band at a molecular weight of 105 kDa that was markedly increased in AD as compared with controls (Fig. 2A, B). This p105 band was also observed in homogenates of cerebral white matter, but there was no difference in its amounts between AD and controls (data not shown). When the pellet portions of cerebral cortex were examined, we found increased staining of both p105 and 45-kDa IκBγ in AD brain as compared with controls (Fig. 2C). In five of six control samples, p105 and IκBγ were hardly detectable. In contrast, except for p105 in one case, both p105 and the 45-kDa IκBγ were clearly stained by anti-IκBγ in AD samples. The better staining of the 45-kDa IκBγ in the pellet fractions than in homogenates was probably due to its enrichment in the pellet fractions. Masliah et al. [9] once reported an association of the nuclear antigen p105 with senile plaques and neurofibrillary tangles in AD brain. This p105 is different from the NF-κB precursor p105, because these two proteins have different primary sequences and the level of the nuclear antigen p105 is not elevated in AD brain [9].

Glycosylation of IκBγ has not been reported previously. The above results suggested that IκBγ might be glycosylated in human brain, but we could not rule out the possibility that the MAA-staining of the 45-kDa band was due to a 45-kDa glycoprotein that co-migrated with IκBγ in SDS-PAGE. Hence, we immunoprecipitated IκBγ from the extract of nuclear fraction of an AD brain with anti-IκBγ immobilized on protein G agarose beads under stringent conditions (20 mM $Na_3PO_4$, pH 7.5, 500 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 0.02% $NaN_3$). This condition eliminated non-specific binding of proteins to the antibody and beads. We found by using Western blots and lectin blot analyses that the immunoprecipitated IκBγ was indeed stained by MAA (Fig. 2D). The MAA-staining was not due to IgG (anti-IκBγ), which itself is a glycoprotein, because no staining was seen with the equivalent amount of anti-IκBγ (Fig. 2D, lane 3).

To elucidate whether the MAA-staining of IκBγ was indeed due to its modification by oligosaccharides terminally linked with sialic acid–(α2,3)-galactose, we treated the blots of human brain cortical homogenates with 0.1 N HCl at 80 °C for 1 h before staining with MAA. This treatment is known to remove terminal sialic acid from glycoproteins and therefore to eliminate staining of the glycoprotein by MAA [10]. We found that the HCl treatment did not abolish or reduce the staining of IκBγ by MAA, although it abolished the MAA staining of most other brain proteins (data not shown). These results suggest that IκBγ does not contain the MAA-recognizable oligosaccharides and that the MAA-staining of IκBγ is probably due to cross-reaction.

IκBγ was reported to have a molecular mass of ∼70 kDa [4,5]. However, it showed an apparent molecular mass of 45 kDa in our studies. It is possible that the 45-kDa MAA-positive IκBγ that was found to be increased in AD brain is a truncated form of IκBγ, but we did not observe a 70-kDa band by either MAA staining or anti-IκBγ staining. Alternatively, human brain might be dominated by a smaller isoform of IκBγ. Smaller isoforms of IκBγ due to alterna-

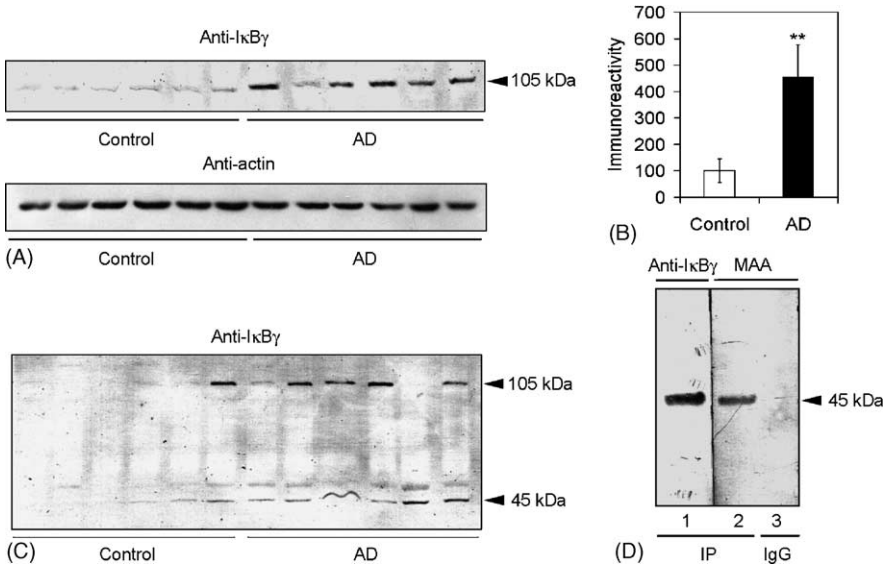


Fig. 2. Identification of the 45-kDa protein as IκBγ. (A) Western blots of homogenates (10 μg/lane) from cerebral cortex of six control and six AD brains stained with anti-IκBγ (1:200 dilution) that was developed by alkaline phosphatase staining using 4-nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indoyl phosphate as substrates (upper panel). A 105-kDa band (marked with arrowhead) was seen. The same blot was re-probed with anti-actin to confirm the equal loading of protein in each lane (lower panel). (B) Densitometric quantitation of the 105-kDa band stained with anti-IκBγ, $^{**}p < 0.01$ vs. controls. (C) Western blots of nuclear fractions (5 μg/lane) from cerebral cortex of six control and six AD brains developed with anti-IκBγ. Arrowheads indicate the 105-kDa NF-κB precursor, p105, and 45-kDa IκBγ. (D) IκBγ immunoprecipitated from the nuclear extract of an AD brain was examined by Western blots developed with anti-IκBγ (lane 1) and MAA (lanes 2, 3), respectively. Lane 3 was loaded with the same amount of anti-IκBγ IgG as was in lane 2.

tive mRNA splicing, IκBγ-1 and IκBγ-2, were previously reported [3].

Our studies demonstrated for the first time the expression of IκBγ in human brain and the elevated levels of both NF-κB precursor p105 and IκBγ in AD brain. The elevation and activation of p65 and p50 of NF-κB pathway have been reported in AD [6,14]. Hence, in addition to the activation of NF-κB, our data indicate that the syntheses of NF-κB precursor and IκBγ are also increased in AD brain. To date, it is not known whether the activation of NF-κB pathway plays active roles in the pathogenesis of AD or merely represents a consequence or response of the disease. Because amyloid β-peptide and oxidative stress, both of which are elevated in AD brain, induce activation of the NF-κB pathway, and the latter is neuroprotective [1,2,6,14], the elevation and activation of the NF-κB pathway may mediate the responses to the pathogenesis and may be involved in the molecular mechanism of AD.

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