[12:20 – 13:00]

# Beta-amyloid peptide degradation by aminopeptidase and its functional role in Alzheimer's disease pathogenesis

**Sangmee Ahn Jo**
**Division of Brain Diseases, Center for Biomedical Sciences**
**National Institute of Health, Korea**

Beta-amyloid peptide (Aβ) is a major component of senile plaques and its aggregation is considered to play a critical role in pathogenesis of Alzheimer's disease (AD). Aggregation of Aβ could result from both increased synthesis and decreased degradation of Aβ. Our laboratory is interested in understading the mechanism of Aβ degradation in brain. Recently our laboratory identified a bacterial gene (SKAP) from *Streptomyces sp* KK565 whose protein product has an activity to cleave Aβ and thus reduce the Aβ-induced neurotoxicity. The sequence analysis showed that this gene was closely related to aminopeptidase. Maldi-Tof analysis showed that the recombinant SKAP protein expressed in E. coli cleaves both Aβ 40 and Aβ 42 at the N-terminal of Aβ while an aminopeptidase from *Streptomyces griseus (SGAP)* cleaves at the C-terminal. We also identified a mammalian homolog of SKAP and the recombinant mammalian protein expressed in Sf-9 insect cells showed a similar proteolytic activity to SGAP, cutting Aβ at the C-terminus. I will discuss the detailed mechanism of the enzyme action and its functional implication in AD.

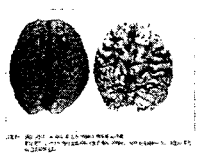
# Beta-amyloid peptide degradation by aminopeptidase and its functional role in Alzheimer's disease pathogenesis

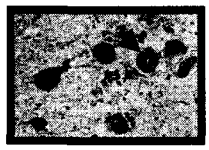
**Sangmee Ahn, Ph.D**

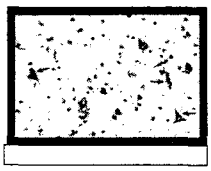
**Division of Brain Diseases**
**Center for Biomedical Sciences**
**and Biomedical Brain Research Center**
**National Institute of Health**

2

## Alzheimer's Disease (AD)







- **Pathology** : senile plaque (β-amyloid peptide), neurofibrillary tangle (tau), neocortical atrophy, neuron and synapse loss
- **Risk factors** : 1) increased age 2) family history
  3) gender 4) head injury
- **Genes involved in AD:**
  **1) Causative genes**: amyloid precursor protein (APP)
  presenilin 1, 2 (PS1, PS2)
  **2) Risk factor genes**: $ApoE_4$, $alpha_2$-macroglobulin ($\alpha_2M$), LRP

2005.12.3

3

# Genes linked to AD

Table 1. Overview of *Established* AD Genes Influencing the Aβ Life Cycle

| Gene (Location [Mb])[a] | Genetic Mechanism | Biochemical Phenotype |
|---|---|---|
| *APOE* (19q13 [50 Mb]) | LOAD: risk association (ε4-allele) | a) ↑ Aβ aggregation<br>b) ↓ Aβ clearance |
| *APP* (21q21 [26 Mb]) | EOFAD: AA-change (*n = 16 mutations*[b])<br>LOAD: mostly neg. association findings | a) ↑ $A\beta_{42}/A\beta_{40}$ ratio<br>b) ↑ Aβ generation/Aβ aggregation |
| *PSEN1* (14q24 [73 Mb]) | EOFAD: AA-change (*n = 140 mutations*[b])<br>LOAD: pos./neg. association findings | ↑ $A\beta_{42}/A\beta_{40}$ ratio |
| *PSEN2* (1q42 [223 Mb]) | EOFAD: AA-change (*n = 10 mutations*[b])<br>LOAD: mostly neg. association findings | ↑ $A\beta_{42}/A\beta_{40}$ ratio |

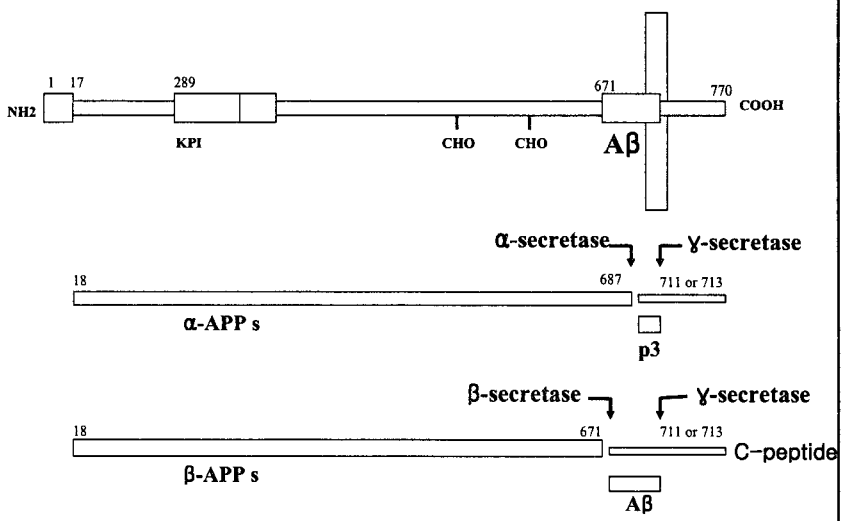
"Mb" = million base-pairs, "EOFAD" = early-onset familial AD, "LOAD" = late-onset AD.
[a]Location according to "UCSC Human Genome Browser," May 2004 assembly (URL: http://genome.ucsc.edu/cgi-bin/hgGateway).
[b]According to the "Alzheimer's Disease Mutation Database" (URL: http://molgen-www.uia.ac.be/ADMutations/).

CELL 120:545-555 (2005)

2005.12.3

4

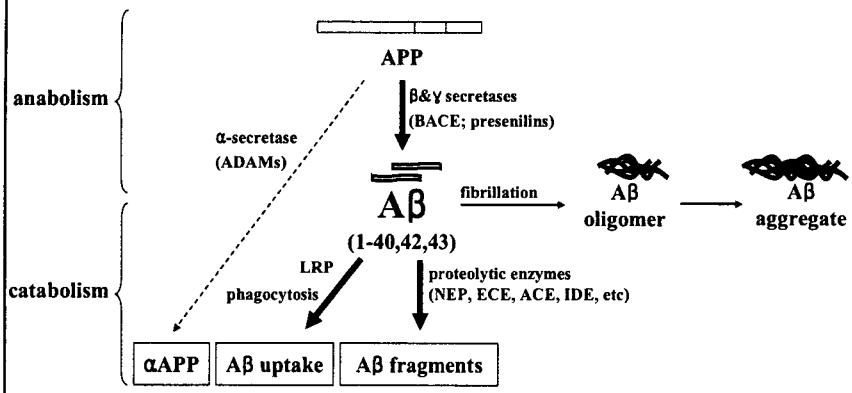
# Amyloid precusor protein (APP) processing




2005.12.3

5

## Amyloid peptide metabolism




2005.12.3

6

## Proteolytic enzymes involved in degradation of the Alzheimer's amyloid peptide

**Neprilysin family**

neprilysin (NEP)
endothelin-converting enzyme (ECE-1)
angiotensin-converting enzyme (ACE)

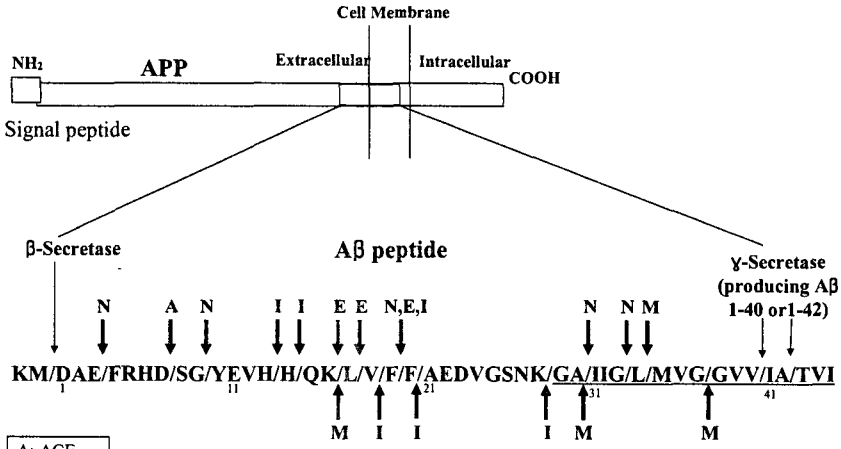
**Insulin-degrading enzyme family**

insulin-degrading enzyme (IDE)

2005.12.3

7

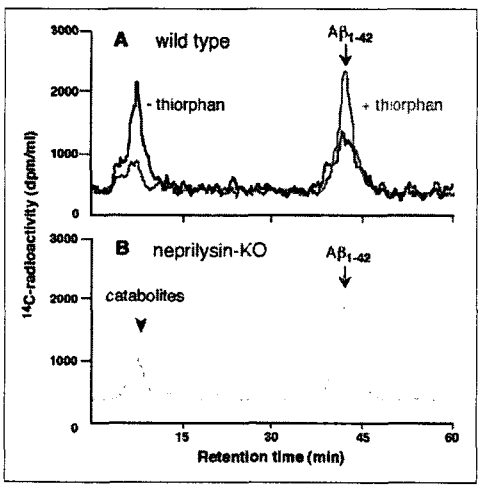
## Peptidases responsible for Aβ cleavage




Julie *et al.*, (2002) J. Neurochem. 81, 2

2005.12.3

## Metabolic Regulation of Brain Aβ by Neprilysin




inhibitors

| | |
|---|---|
| NEP | thiorphan, phosphoramidon |
| ECE | captopril, phosphoramidon |
| IDE | ? |

*Saido group., Science(* 2001) 292:1550-1552

9

## Screening of microbial secretion product

- *Identification of the candidate compounds* from microbacteria, fungi and seeweeds libraries that are able to block ***Aβ-aggregation***

2005.12.3

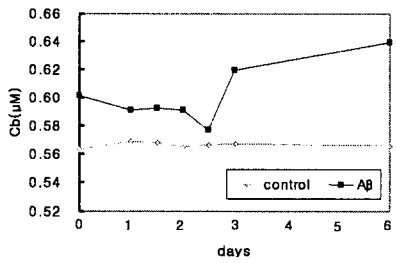
10

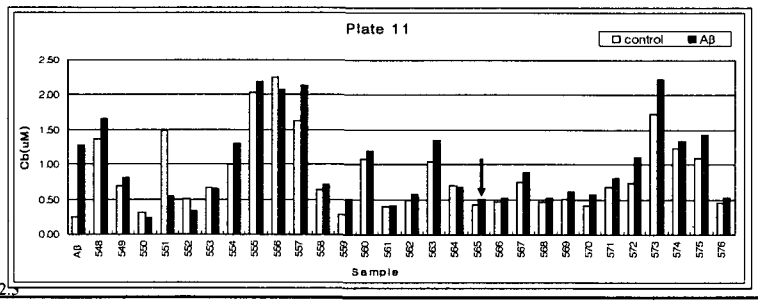
## Amlyoid peptide aggregation assay

**1. Congo red Assay**
**Cb[M]=($A_{540}$/25.295)-($A_{480}$/46.306)**

**2. Thioflavin T assay**

**3. Observe the pallet formation following centrifugation**

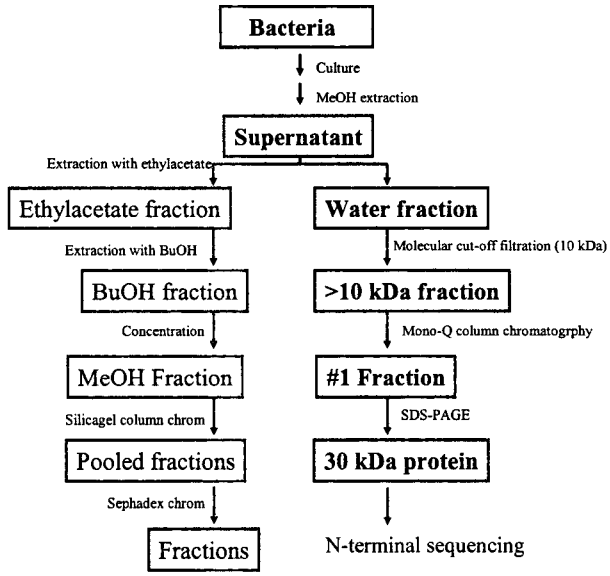



2005.12.3

11

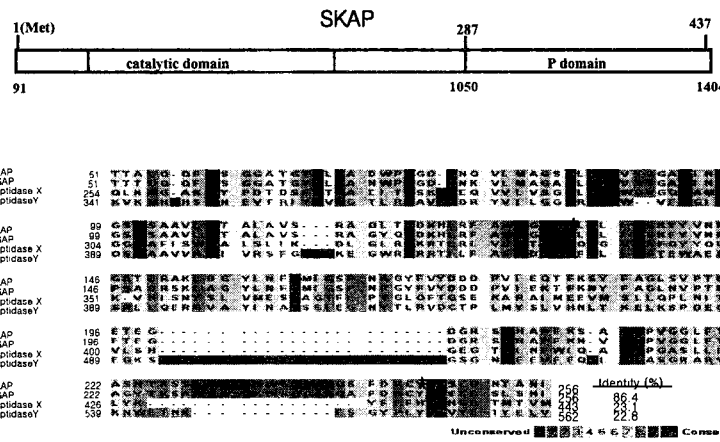
# Purification steps




2005.12.3

12

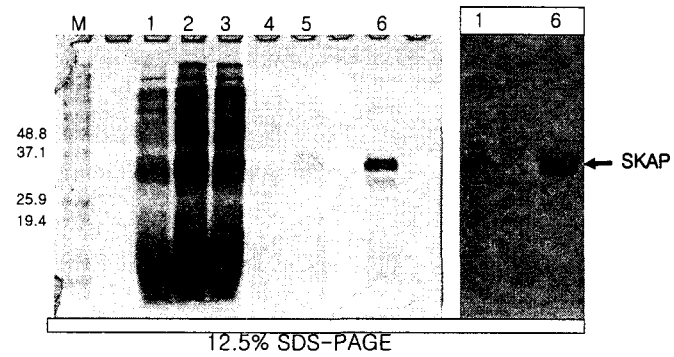
# Amino acid sequence comparison with other proteases




2005.12.3

13

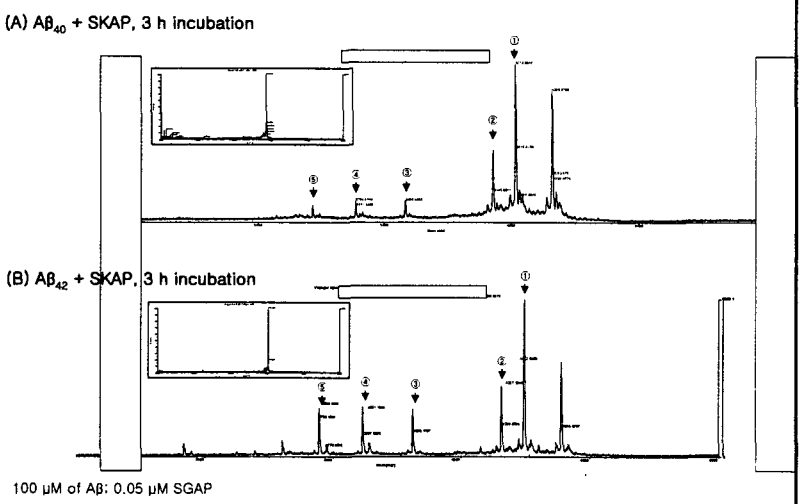
**Preparation of the recombinant protein, SKAP30kDa, and identification with anti-SKAP antibody**




2005.12.3

14

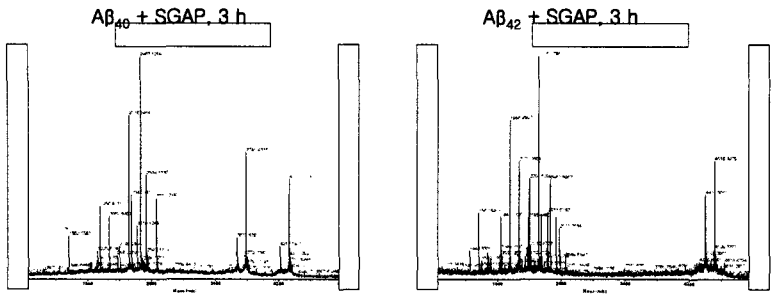
**Amyloid peptide$_{40, 42}$ cleavage by SKAP: Maldi-Tof analysis**




2005.12.3

15

## Amyloid peptide $_{40, 42}$ cleavage by SGAP

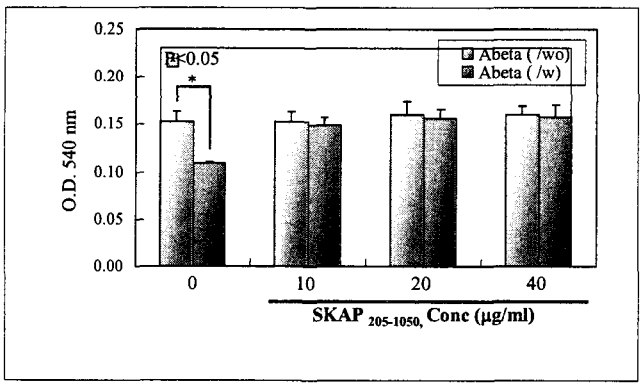



100 μM of Aβ : 0.05 μM SGAP

2005.12.3

16

## Inhibition of Aβ-induced neurotoxicity by SKAP




2005.12.3

17

## Summary

- We purified and cloned a gene (SKAP) from *Streptomyces* sp. (#90565) whose product is able to cleave amyloid peptide.

  *Coding region=1314 bp, 437 amino acid, M.W.=45,209*

- Recombinant SKAP protein (30 kDa) blocked both fibrillation of amyloid peptide and reduced amyloid peptide–induced neuronal toxicity.

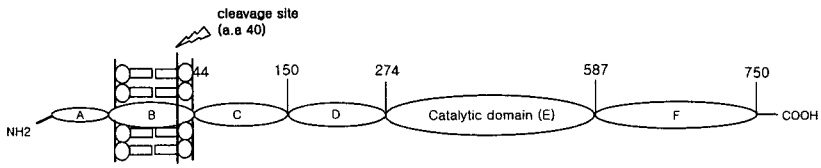
2005.12.3

18

## Peptidase X

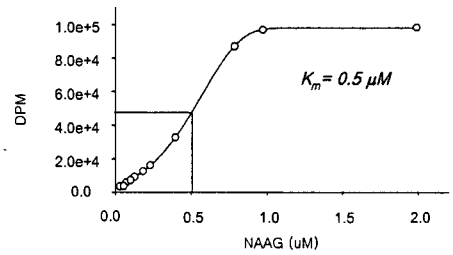
- **90-100 kDa transmembrane protein**
- **Located on neuronal and glial surfaces**
- **Tissue specificity: in various tissues**

Schematic domain structure of human peptidase X



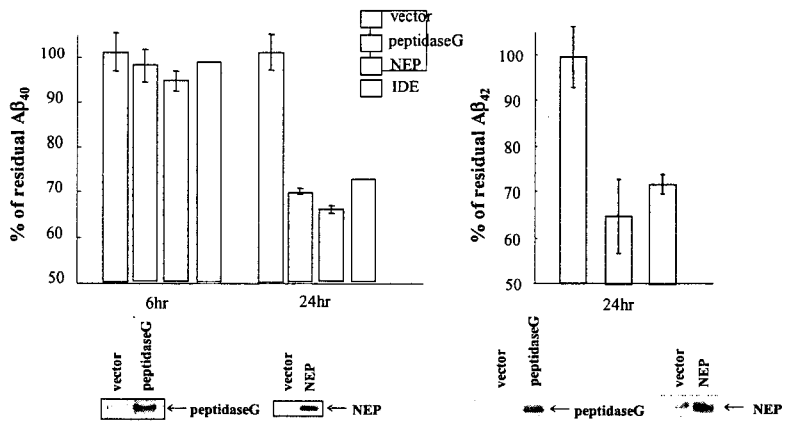

2005.12.3

## Preparation of recombinant peptidase G and measurement of its activity



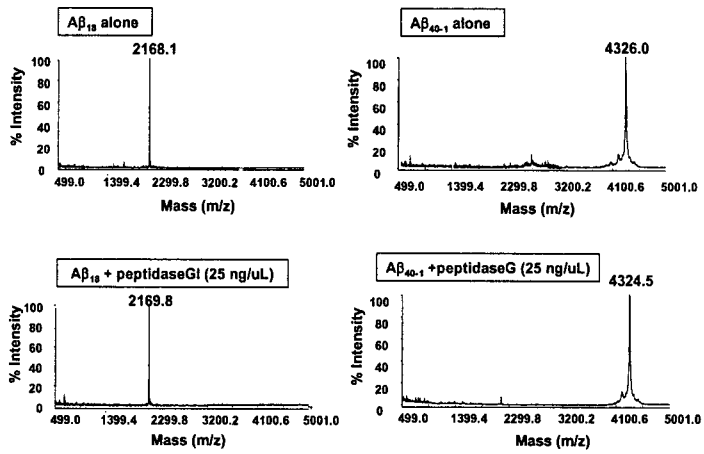

## $A\beta_{40}$ degradation by peptidaseX, NEP, IDE:

**Transient transfection**



21

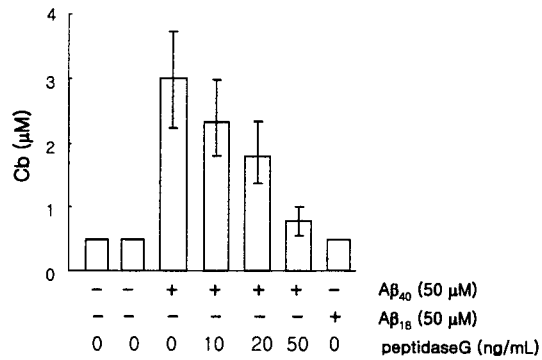
## $A\beta_{40-1}$ and $A\beta_{1-18}$ are not cleaved by peptidase G.




2005.12.3

22

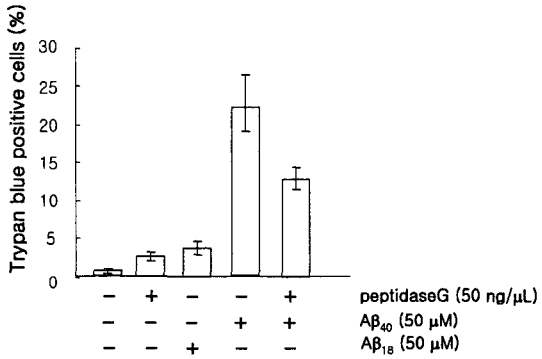
## $A\beta_{40}$ aggregation is reduced by recombinant peptidaseG protein




2005.12.3

23

## Neuronal cell death induced by $A\beta_{40}$ decreased by incubation with peptidase G




2005.12.3

24

## Summary

- Both synthetic and endogenous Aβ are degraded by peptidase G.
- Both Aβ40 and 42 are cleaved by peptidase G.
- Peptidase G cleaves Aβ40 into small fragments (Aβ18) which lacks aggregation property and are not toxic to neuron.
- Peptidase G seems to degrade multimeric Aβ more efficiently than monomeric Aβ.
- Peptidase G protects neurons from toxicity induced by Aβ by cleaving it into smaller fragments.
- Thus, dis-regulation of peptidase G could contribute amyloid deposit found in AD brain.

2005.12.3

25

## Ongoing project with aminopeptidase G and its future application to AD therapy

- Treatment of AD mouse model (APP and presenilin) with peptidase G inhibitor: increases the both Ab40 and 42 level.
- Gene delivery of peptidaseG into the brain of AD model mouse
- Identification of the pharmacological agents that increase the endogenous peptidaseG activity
- Identification of the transcriptional factors or the signaling pathways that stimulate transcription of the peptidaseG gene

2005.12.3

26


**Div. of Brain Diseases, NIH, Korea**
**Sangmee Ahn, Ph.D**

SKAP:
Yoo Chul Bae, MS
Park Jeong Eun, Ph.D

PeptidaseG:
Kim, MinJu, MD, PhD
Ahn, Kyungsook, Ph.D
Soyoung Suh, Ph.D
Chae Sansook, BS
Jang Bong Kum, MS
Hyuck Kim, MS
Chulman Jo, Ph.D

**Center for Biomedical Sciences, NIH, Korea**
**Inho Jo, Ph.D**

Shin-Hyun Joo, MS
Kim Eun Kyung, MS

***Thank you!!***

**Samsung Medical Center**

**Kim Doh Kwan, MD, PhD**

**Ansan Korea University**
**Dept. of psychiatry**

**Han Chang Soo, MD, PhD**


2005.12.3