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Protective effect of a 3 kDa peptide obtained from beef myofibrillar protein using alkaline-AK on neuronal cells
(#92)

Seung Yun Lee, Da Young Lee, Hea Jin Kang, Rana M. Shohel, Sun Jin Hur

Chung-Ang university, Department of Animal Science and Technology, Anseong-si, South Korea

Introduction
With the increase in the aging population, the prevalence of a number of aging neurodegenerative diseases, such as Alzheimer's disease (AD), and Parkinson's disease (PD) is on the rise.

Various natural peptides derived from many food protein hydrolysates exhibit several beneficial activities, such as antioxidant, neuro-protective or memory impairment, anti-hypertensive, anti-inflammatory, and antimicrobial activities Several studies have also demonstrated a close association between the prevention of neurodegenerative diseases and antioxidant activity.

However, the protective effect of peptides with antioxidant activity, derived from beef myofibrillar protein using inexpensive enzymes, such as alkaline-AK, on neuronal cells have not been reported extensively. Moreover, the mechanism by which these peptides exert neuroprotective effects against oxidative stress-induced neuronal cells is not known. Therefore, the aims of this study were to 1) determine the antioxidant potential of peptides with a molecular weight of less than 3 kDa, obtained using an inexpensive enzyme, such as alkaline-AK, by performing *in vitro* radical scavenging assays, 2) to evaluate their neuroprotective effects against H_2O_2-induced apoptosis in human neuronal SH-SY5Y cells, and 3) and to identify the peptide sequences.

Methods
These peptides were isolated and further separated by fast protein liquid chromatography (FPLC), and their protective effect against H_2O_2-mediated cell death was measured by determining cell viability, nitric oxide (NO) production, mitochondrial membrane potential (MMP), apoptosis, morphological changes in cell nuclei, and in vitro antioxidant assays.

Results
The ABTS radical scavenging activity of both the AK3K peptide fractions (AK3KF1 and AK3KF2) was more than 70%, however, AK3KF1 exhibited a significantly higher ABTS radical scavenging activity than did the AK3KF2 fraction. Furthermore, AK3KF1 had a significantly higher iron chelating activity, nitrite scavenging activity, and reducing power than did the AK3KF1 fraction.

Treatment with 0.25 and 0.5 mg/mL AK3KF1 and AK3KF2, respectively, significantly increased ($p<0.05$) cell viability compared to the H_2O_2 treated SH-SY5Y cells. Additionally, treatment with 0.25 and 0.5 mg/mL concentration of AK3K peptide fractions (AK3KF1 and AK3KF2) inhibited NO production in SH-SY5Y cells compared to the H_2O_2 treated cells ($p<0.001$).

Treatment with both the AK3K peptide fractions exhibited protective effects on neuronal cells by reducing cell shrinkage and nuclei condensation compared to the H_2O_2 treated neuronal cells. Furthermore, nuclear condensation was found to be lower in cells treated with AK3KF1 than in the cells treated with AK3KF2.

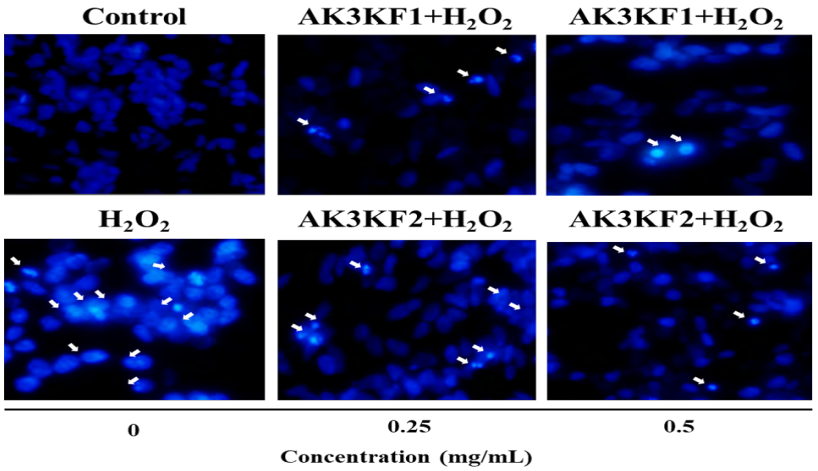
We observed that MMP (JC-1 aggregates/monomer) decreased in H_2O_2 treated cells, and treatment with 0.5 mg/mL AK3KF1 significantly increased MMP compared to that of the H_2O_2 treated cells ($p < 0.05$). These results indicated that AK3KF1 reduced H_2O_2 induced neuronal cell damage and restored MMP.

Treatment with 0.2 mM H_2O_2 increased the percentage of apoptotic cells to 86.80 ± 10.33 %, which decreased to 18.82 ± 19.77 % and 43.32 ± 16.94% in the presence of AK3KF1 and AK3KF2 peptide fractions, respectively. The AK3KF1 fraction showed a significantly stronger neuroprotective effect compared to the AK3KF2 fraction in H_2O_2treated neuronal cells ($p < 0.05$). These results indicate that AK3KF1 protected neuronal cells against H_2O_2 induced oxidative stress.

Since our results confirmed that the AK3KF1 had significantly high antioxidant potential and exhibited a stronger neuroprotective effect. The amino acid sequence of AK3KF1 was analyzed using the LTQ Orbitrap XL mass spectrometer. The sequence of the peptide was identified as Thr-Gln-Lys-Lys-Val-Ile-Phe-Cys with a molecular weight of 965.54 Da.

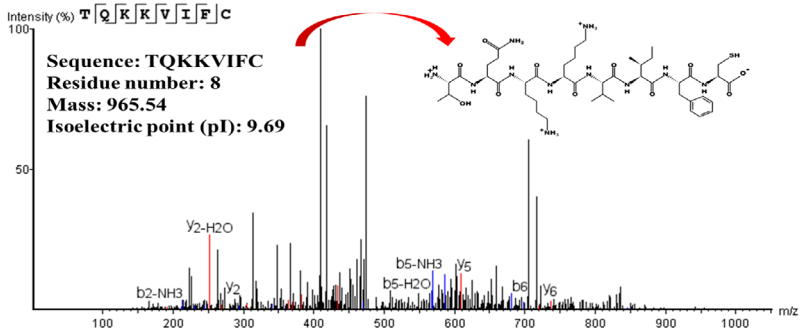
Conclusion
This study demonstrated the neuroprotective effect of two <3 kDa peptide fractions (AK3KF1 and AK3KF2) obtained from beef myofibrillar protein using an inexpensive enzyme (alkaline-AK) on human neuronal SH-SY5Y cells. The two peptide fractions (AK3KF1 and AK3KF2) showed neuroprotective effect by increasing cell viability, inhibiting NO production and fragmentation of cell nuclei, increasing MMP, reducing apoptosis, and ability to scavenge free radicals. Furthermore, AK3KF1 exhibited stronger neuroprotective effect than the AK3KF2 fraction. The sequence of AK3KF1peptide was identified as Thr-Gln-Lys-Lys-Val-Ile-Phe-Cys with a molecular weight of 965.54 Da. The neuroprotective effect of this peptide could possibly be related to amino acid composition, hydrophobicity, and ability of peptides to penetrate through the cell membranes.

Notes



Morphological assessment of AK3K fractions on hydrogen peroxide treated neuronal cells

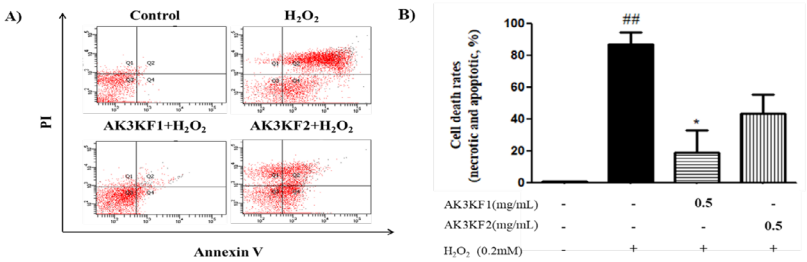
Effect of 0.25 and 0.5 mg/mL concentration of 3 kDa peptide fractions obtained from beef myofibrillar protein using alkaline-AK on fragmentation of nuclei by Hoechst staining in H2O2-treated human neuronal SH-SY5Y cells. Data are given as mean±standard deviation (n=3).



Identification of amino acid sequence of AK3K fraction

Identification of molecular mass and amino acid sequence of AK3KF1 fraction. MS/MS was performed on a LTQ Orbitrap XL mass spectrometer.

Notes



Neuroprotective effect and cell death rate of AK3K fractions

A) Flow cytometry analysis, and B) quantitative analysis of the cell death rate to determine protective effect of 3 kDa peptides on neuronal cells. ## p<0.001 compared to the control cells. *p<0.05 compared to the H2O2-treated cells.

Notes