

D10

## **NMR Analysis of Pure Separated Water-Soluble *B. mori* Fibroin Fractions and Acetylcholine Levels in Brain of SD Rats**

**Joo-Hong Yeo<sup>1</sup>, Kwang-Gill Lee<sup>1</sup>, HaeYong Kweon<sup>1</sup>, Soon-Ok Woo<sup>1</sup>,  
Sang-Mi Han<sup>1</sup>, Yong-Woo Lee<sup>1</sup> and Makoto Demura<sup>2</sup>**

<sup>1</sup>*Department of Agricultural Biology, National Institute of Agricultural Science and Technology, Suwon 441-100, Korea and* <sup>2</sup>*Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan.*

The silk fibroin powder has been widely used as industrial materials such as biomaterials, cosmetics and foods etc. since various materials; fiber, film, gel and powder can be made from this protein. It is known that the *B. mori* fibroin forms molecular complex consisting of heavy (H)-chain of 390 kDa and two lower molecular mass protein components: fibroin light chain (L-chain) of 26 kDa and P25, which is a glycoprotein of about 30 kDa. Recently, the complete primary structure of *B. mori* fibroin H-chain (5,263 residues, main component of the silk fibroin) has been reported. We have previously reported pure-separation of calcium chloride-treated silk fibroin hydrolysates by gel filtration chromatography and effect of enzymatic digestion in order to prepare functional silk fibroin powder. In this study, nuclear magnetic resonance (NMR) spectra of pure separated silk fibroin powder dissolved in water was observed to characterize the amino acid composition and the structural details. Two kinds of water soluble silk powders hydrolyzed with hydrochloric acid and enzyme were dissolved in 99 % deuterium oxide for NMR measurements. <sup>13</sup>C and <sup>1</sup>H NMR spectra of these samples were compared using several NMR pulse sequences to assign NMR peaks. In the case of the silk hydrolysate prepared by hydrochloric acid treatment and gel filtration chromatography, <sup>13</sup>C peaks attributed to each carbon of only main amino acid composition were observed. Contrary, the NMR peaks of the silk fibroin powder made from enzyme digested peptide shows more complex pattern compared with the former powder.

On the other hand, the effect of acetylcholine levels in rats were considerable increase (8.9% and 13.9%, respectively) in brain membranes of these two samples compared with control group.