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NMR Analysis of Water-Soluble Silk Fibroin Powder

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The non-textile materials made from silk proteins have been widely studied and used in biomaterials, cosmetics and foods etc. since various materials such as fiber, film, gel and powder can be made from this protein, especially the silk fibroins. It is known that the *Bobmyx 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 (5263 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 the present study, nuclear magnetic resonance (NMR) spectra of the silk fibroin powder dissolved in water was observed to characterize the amino acid composition and the structural details. Two kinds of the silk powders hydrolyzed with hydrochloric acid and enzyme were dissolved in 99 % deuterium oxide for NMR measurements. ^{13}C and ^{1}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, ^{13}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. Detailed structural characterization of the silk fibroin powders based on NMR analysis was discussed.